The Journal of Comparative Pathology and Therapeutics

Vol. 61. No. 2. Price 8 shillings and sixpence

1. The columns of the Journal are open for contributions recording original research in veterinary science.

2. The Editorial Board accepts papers bearing on the above subject on the understanding that they are not being offered elsewhere for publication. It reserves the right to reject any papers submitted to it for publication.

3. It is essential that all articles should be concise. Any necessary allusions to matter already published should be brief and limited strictly to those relevant to the satisfactory exposition of the author's own work.

4. Citation of literature in the text should be accompanied by the year of publication. Authors' names (followed by year, publication, etc.) should be arranged alphabetically in a list at the end of the paper.

5. Papers for publication, exchange journals, etc., should be sent to Prof. T. J. Bosworth, Royal Veterinary College, London, N.W.1 or to any member of the Editorial Board.

6. Correspondence concerning business matters should be addressed to the Publishers, The University Press of Liverpool, Liverpool, 3.

7. The net price of each volume of four numbers is £1 10s. paid in advance to the publishers. Single numbers may be obtained at a price of 8s. 6d. each.

Additional copies and reprints ordered before the issue is printed may be obtained from the publishers.

CONTENTS

PAGE

Studies on Experimental Streptococcal Mastitis. V. Histological Findings in Experimental Streptococcal Mastitis in the Goat. By I. H. Pattison 71

The Experimental Production of Bracken "Poisoning" in Sheep. By F. E. Moon and M. A. Raafat ... 88

Studies on Respiratory Diseases of Pigs. III. Persistence of Influenza Viruses in the Respiratory Tract. By T. S. Gulrajani 101

Studies on Respiratory Diseases of Pigs. IV. Transmission of Infectious Pneumonia and its Differentiation from Swine Influenza. By T. S. Gulrajani and W. I. B. Beveridge 118

Canine Chronic Nephritis. I. Observations on the Pathology of the Kidney. By H. Platt 140

A Note on a New Method for Preparing a Potent Anti-Chauvoei Vaccine. By M. Sterne, P. Thorold, and J. R. Scheuber 150

Leptospira Icterohaemorrhagiae Infection in Pigs. By D. I. Nisbet 155

STUDIES ON EXPERIMENTAL STREPTOCOCCAL MASTITIS

V. Histological Findings in Experimental Streptococcal Mastitis in the Goat

By

I. H. Pattison.

Agricultural Research Council Field Station, Compton, Berks.

INTRODUCTION

The previous papers in this series (Pattison, Taylor and Holman, 1950; Taylor, Holman and Pattison, 1950; Holman, Pattison and Taylor, 1950; Pattison and Holman, 1951) were concerned with a description of the disease produced by inoculating *Str. agalactiae* strain S 13 into the teat canal of goats. All the animals used in those experiments were killed for histological examination of the udder, and in this present paper the results of this examination are recorded.

MATERIALS AND METHODS

Details of the material examined are given in Table 1; all animals were inoculated into one side of the udder only, but sections were always prepared from both sides. The number of blocks of tissue examined is given because the very patchy nature of the disease makes it necessary to indicate the scope of the examination on which conclusions have been based. A block of tissue was about 15 mm. square and 5 mm. thick, and two or more sections were examined from all blocks.

Various fixatives were tried, of which ten per cent. formalin containing four per cent. by weight of sodium acetate was found satisfactory. Udders were removed immediately after destruction of the animal and were fixed by perfusion through the mammary artery on each side; after injection of the arteries, fixative was pumped into the teat canals and the whole udder was then suspended in fixative for several days. This method of fixation removed most of the blood from the tissues and dilated the blood vessels to a certain extent; pathological dilation of vessels and ducts was estimated by comparison with the non-inoculated side of the udder. After fixation, udders were sliced dorso-ventrally, parallel with the long axis of the body, and were stored in two per cent. formalin in thirty per cent. alcohol. Slices required for histological examination were photographed (see Fig 52), and the position of origin of all blocks was marked on a full-size drawing of each slice; the block surface to be cut was marked with a notch.

Several methods of preparing blocks were tried and the following was found to be reliable. The tissues were dehydrated in alcohols of increasing strength, cleared in benzole and embedded

TABLE 1

DETAILS OF MATERIAL EXAMINED.

Type of inoculum	*Time between inoculation and destruction*	*No. of goats*	*No. of blocks examined from :—*	
			Inoculated side	*Non-inoculated side*
Heat-killed streptococci	6 hours	1	24	2
,,	9 days	2	12	2
,,	45 ,,	1	14	1
Living streptococci	6 hours	4	53	8
,,	12 ,,	1	26	1
,,	24 ,,	2	28	9
,,	2 days	2	25	2
,,	3 ,,	1	19	1
,,	4 ,,	1	6	1
,,	7 ,,	1	23	4
,,	9 ,,	9	271	51
,,	13 ,,	1	21	1
,,	15 ,,	2	47	3
,,	18 ,,	1	8	1
,,	22 ,,	1	27	2
,,	27 ,,	1	23	1
,,	36 ,,	1	15	1
,,	45 ,,	1	15	6
,,	54 ,,	1	16	1
,,	60 ,,	1	19	2
,,	63 ,,	1	13	1
,,	71 ,,	1	18	1
,,	80 ,,	1	10	1
,,	82 ,,	1	8	2
	TOTAL	39	741	105

after two changes of 58° C. wax in a vacuum embedder. Sections were cut on a sliding microtome. One section from each block was stained by haematoxylin and eosin, and one by Gram's method. Other staining methods used were van Gieson, Giemsa, Vierhoeff, and lissamine fast red for myoepithelium.

Figure 53a was prepared by the method described by Shennan (1929). All photographs were of tissue stained by haematoxylin and eosin.

RESULTS

The mammary gland of the normal goat

The histological structure of the mammary gland of the goat has been described by Turner (1939) and appears to be closely similar to that of the cow.

In examining the mammary gland of the goat for pathological alterations, the marked variations that may occur in the normal picture must be borne in mind. The stage of lactation has an important influence on histological structure (Figs. 1, 2 and 3);

and the picture of the milked-out gland (Fig. 4) differs from that of the gland distended with secretion at the time of death (Fig. 5). The number of pregnancies probably influences the histological picture to some extent and there can be quite marked variations in different areas of the same gland (e.g. Figs. 1 and 4 are from the same animal).

The occurrence of *corpora amylacea* in the normal bovine mammary gland was discussed by M'Fadyean (1930), and Morrill (1938) drew attention to their more frequent occurrence in the later stages of lactation. These bodies were found in normal goats also (Figs. 3 and 6) and were most numerous in late lactation.

Attention has been drawn by Bryan (1946) to the development of caprine acinar epithelium into finger-like processes, the tips of which are discharged into the acinar lumen and excreted with the milk. This type of epithelium was noted in many sections (Fig. 7). Richardson (1947) has expressed doubt concerning this "decapitation" of secreting cells and has suggested the possibility that artefact might be involved.

In some sections the gathering of acinar epithelium into "bunch-of-grapes" clusters was noted (Fig. 8); usually the acinar epithelium of an entire lobule, or of two or three adjacent lobules, showed this formation, while in the remainder of the section acinar epithelium was of the usual type. The typical lining epithelium of a large duct in mid-lactation is shown in Figure 9, but in some sections duct epithelium was secretory in character (Fig. 10); the occasional occurrence of secretory duct epithelium in the cow was noted by Turner.

Small foci of lymphocytes were occasionally found in sections of normal tissue (Fig. 11), and on rare occasions small accumulations of homogeneous cosinophilic material with a few neutrophils were found in sections from sides of udders not inoculated with streptococci (Fig. 12).

Dawson (1935) described the presence of non-secreting lobules in the normal human breast. Non-secreting (i.e. pre-functional) lobules were also found in the fully lactating goat udder (Fig. 13), but they were not usually numerous and were seldom as well defined as in the case illustrated.

McFarlane and Rennie (1950) said that involuted tissue may be found in the lactating bovine mammary gland. However, they did not specify the stage of lactation of glands in which involuted tissue occurred and did not state their criteria for identification of such tissue. I have been unable to find involuted lobules in normal goats in early or in full lactation. Examination of udders in late lactation showed that involution was a slow and rather patchy process, but even in late lactation I did not find single involuted lobules surrounded by normal lactating tissue; such a finding was confined to some udders inoculated with streptococci. The material examined by McFarlane and Rennie was obtained

from 25 cows "showing the least possible degree of palpable mastitis . . . at the abattoir". A full clinical history of these animals was not apparently available. In these circumstances, it is possible that the presence of involuted tissue in quarters that showed no clinical abnormality may have been due to a previous—perhaps unsuspected—inflammatory process.

In this study, all goats examined histologically had been inoculated into one half of the udder only. In every case the availability of non-inoculated tissue for comparison with the abnormal was found to be of great assistance in assessing pathological changes attributable to experimental procedure.

The mammary gland of the goat in mastitis

The patchy nature of the disease. The following description of the disease caused by the inoculation of *Str. agalactiae* into the teat canal of goats is based on examination of sections from animals in many different stages of the disease. An important finding that must be strongly emphasised is that a greater or lesser amount of *normal* tissue was found in practically every inoculated udder examined, and that varying degrees of tissue reaction could invariably be demonstrated in different areas of the same microscopic section and in different sections from the same udder. Small areas of early reaction to infection could almost always be found in animals showing predominantly the later pathological changes of the disease. This finding is in agreement with the observations of Morrill, and others, in cases of bovine streptococcal mastitis.

Comparison of results in goats with reported findings in bovine streptococcal mastitis. Pattison and Holman (1951) expressed the opinion that the disease produced in goats, as described in earlier papers in this series, was not significantly different from naturally occurring bovine streptococcal mastitis as described in the literature. Histopathological examinations have strengthened this opinion. In the available literature on the histopathology of bovine streptococcal mastitis, I have been unable to find a photographic illustration that could not be duplicated in material from the experimental disease in the goat. It seems justifiable, therefore, to consider the findings in the goat in the light of what is already known of the disease in the cow.

Histological Examination of Animals Inoculated with Heat-killed Streptococci

Four udders inoculated with heat-killed organisms were available for examination (see Table 1). In the animal killed six hours after inoculation there was a very slight migration of neutrophils into occasional secreting acini. The two animals killed at nine days were in a late stage of lactation and involutionary changes were well marked in the non-inoculated sides of both udders; in both cases it appeared that involution was slightly more advanced

in the inoculated side than in the non-inoculated side, but no other difference between the two sides was detected. No abnormality was found in the inoculated side of the animal destroyed 45 days after inoculation.

Histological Examination of Animals Inoculated with Living Streptococci

Pattison and Holman classified the types of reaction to inoculation with living streptococci as (a) marked (b) intermediate (c) minimal, and described each in detail. This classification has been found to have a sound histopathological basis. The outstanding difference between marked and minimal reactions was that in the marked reaction the usual initial rise in body temperature within a few hours of inoculation was followed, within the succeeding 21 days, by one or more marked additional rises in body temperature accompanied by some systemic disturbance; whereas in minimal reactions only slight rises in temperature subsequent to the usual "inoculation rise" were detected and there was no systemic disturbance. These later marked rises in temperature have been correlated with a rise in viable count of streptococci per ml. of milk, with an alteration of mouse-lethal power of the streptococci, and with the occurrence of viable streptococci in the supramammary lymphatic gland of the inoculated side of the udder (see previous papers). Histological examination of animals killed during these periods of "crisis" (that may be of only a few hours' duration) has shown that at these times streptococci are penetrating the lining epithelium of the acini and ducts and in two cases chains of Gram-positive streptococci were found in enormously dilated lymphatic vessels.

I have come to the conclusion that these periods of crisis are followed by development of the classical disease known clinically as streptococcal mastitis and that unless a period of crisis, i.e. of extensive penetration of streptococci through the epithelial lining of the duct system occurs then the streptococcus is merely carried within the duct system in a state of equilibrium with its host.

Intermediate reactors to inoculation were detectable histologically as animals that had passed through one or more crises, but in which only a relatively small area of udder tissue had been involved. The pathological changes here were those of the marked reaction, but with a greater area of tissue left undamaged.

For these reasons it is convenient to describe the findings in animals inoculated with living streptococci under two headings; (1) minimal reaction, (2) marked reaction.

Minimal reaction to inoculation of living streptococci

Examination of sections from infected animals killed at various times after inoculation but before a period of crisis had been detected, showed a patchy migration of neutrophils into secreting

acini and a rather more generalised increase in interacinar cellularity, the cells being mainly macrophages and fibroblasts. As the time between inoculation and examination was extended, e.g. at 45 days after inoculation, the neutrophil reaction was less marked, but the increase in interacinar cellularity was more obvious. Some of the larger ducts showed a slight periductal cellular reaction of the macrophage type. Streptococci were usually rather hard to find, but could be identified in the smaller pockets of larger ducts; their presence was always associated with a few cells, or cell debris, loose in the duct lumen. Here and there, streptococci apparently adherent to the surface of intact duct epithelium were found, and in some fields the organisms had the appearance of being just within the cell membrane. No organisms were identified in the interacinar or interlobular tissue. The same abnormalities occurred in some marked and intermediate reaction animals in areas of the udder not involved in the more advanced disease process.

In many respects there is a striking similarity between these findings and those of Andberg and Weirether (1943, 1944) who made a histological examination of normal bovine udders inoculated *via* the teat canal with Novoxil and Tyrothricin. Again, it has been noted above that the inoculation of heat-killed streptococci was followed by a slight migration of neutrophils into secreting acini. My conclusion is that these neutrophil, macrophage and fibroblast reactions constitute a non-specific response to the inoculation of foreign material (probably of any type) into the duct system, and that the differences in extent and intensity between the reactions are quantitative only.

Marked reaction to inoculation of living streptococci

For the sake of clarity, it is convenient to divide a description of this type of reaction into two parts (*a*) changes in the lobules (*b*) changes in the ducts. This reaction is that generally referred to clinically as streptococcal mastitis, beginning with the cardinal signs of inflammation in the udder, varying in degree in individual cases and ending in extreme cases, after a longer or shorter interval, with an atrophied, almost functionless, organ.

Changes in lobules

The first change noted in cases of marked reaction to inoculation was a patchy, but rather extensive, migration of neutrophils out of the blood vessels into the surrounding tissues. In places, enormous numbers of neutrophils were involved in this migration and were found accumulated in the interlobular tissue (Fig. 14) passing directly through the vascular endothelium, the basement membrane and acinar walls into secreting acini (Fig. 15). This period of neutrophil migration corresponded with a dramatic fall in neutrophil count of the peripheral blood and a sharp rise in neutrophil count in Breed smears of the milk, as reported previously. At the same time there was evidence, in places, of a

macrophage and fibroblast reaction in the interacinar tissue. Cocci were not readily found, but occurred here and there associated with clumps of cells in the larger ducts.

Within a short time (and visible in some animals killed six hours after inoculation), the macrophage-fibroblast reaction had increased in extent and intensity and there were marked changes in the acinar epithelium that had a bubbly, vacuolated appearance. In places, this epithelium was heaped up to give the acini a ragged appearance and beneath this abnormal epithelium were macrophages and fibroblasts in considerable numbers. In sections showing these changes streptococci were very numerous. They were present in the contents of large and small ducts, and of many acini, and they were also found in many places deeply embedded in the epithelium and on the inner side of the basement membrane. In such sections there was an astonishing dilatation of lymphatic vessles, many of which showed a macrophage-neutrophil reaction in their walls (Fig. 16). Many of these dilated lymphatics contained numerous neutrophils and these cells occurred in large numbers in the medullary region of the supramammary gland (Fig. 17).

I believe that this histological picture shows penetration of streptococci through the lining epithelium of the duct system and that this process is essential in creating clinical streptococcal mastitis. Extensive involvement of the udder in this change elicits a systemic response from the animal, detectable clinically by a rise in temperature and sometimes by dullness and inappetence. All the evidence indicates that this period of penetration is brief, being measured usually in hours, and that the invading organism is rapidly destroyed. It seems likely that this is the fundamental pathological process in what is called a "flare-up" of streptococcal mastitis in an animal known to be carrying streptococci in its udder. The stimulus required by the streptococcus to enable it to penetrate intact epithelium is unknown. But the facts that a high viable count per ml. of milk is associated with the process and that inoculation of a very large number of living streptococci is followed by epithelial penetration suggest that the udder defences can be overwhelmed by a sufficiently large number of invading organisms. It follows, therefore, that penetration will occur if conditions become favourable for extensive multiplication of streptococci within the udder.

Streptococcal mastitis has been described by Morrill, and by others, as a progressive disease. Histological examination of the condition in goats has made this abundantly clear. It would appear that a group of lobules may remain unattacked by the invading organism and histologically normal for a long period, and then some stimulus causes extension of the disease process within this strictly limited area. Thus, all stages of the disease may be found in the udder of the same animal. An example is shown in Figure 18, where a single lobule in an otherwise almost completely atrophied

udder has become involved in the earliest, neutrophil-migration stage of the disease process.

Once penetration by cocci of the epithelial lining of the duct system had occurred, histological examination showed that the disease proceeded rapidly. Macrophages and fibroblasts were found in increasing numbers in the interacinar tissue (Fig. 19) and in many areas the acini which had a rounded, dilated appearance, contained stringy, eosinophilic material together with intact cells and cell debris (Fig. 20). The macrophage-fibroblast reaction continued until many acini were over-run and obliterated by a mass of cells (Fig. 21). In areas showing this type of change, cocci were found in the contents of larger ducts and occasionally in the contents of acini, but in many sections they were not numerous. These changes were found in some cases within four days of inoculation. Many lymphatic channels were greatly dilated at this time and neutrophils were still numerous in the medullary region of the supramammary gland. In some sections small, dense foci of lymphocytes were found in the interacinar tissue.

Up to this stage it appeared that the essential disease process was a rapidly progressing macrophage-fibroblast reaction, together with damage to and desquamation of, much acinar epithelium. At this stage I believe that attacked lobules began to involute, and that all subsequent stages in the disease were represented histologically as a combination of two processes, pathological fibrosis (*i.e.*, the formation of post-inflammatory scar tissue) and physiological involution. Involution of the normal caprine mammary gland has been studied by Turner and Reineke (1936) and there is little doubt that some of the changes described by them (and that have been noted in animals examined to confirm their findings) were detectable at a very early stage in the disease process. That this involution was initiated by inoculation of streptococci, was confirmed by the fact that involuted tissue was not found in the non-inoculated side of the udder of the animal under examination.

Involution was first detectable as stasis of secretion in widely dilated acini; the retained secretion stained darkly and contained cellular debris and a few neutrophils, the interacinar tissue being uncertainly defined by reason of its cellularity (Fig. 22). Cocci were very rarely seen in these areas and were only relatively numerous in the contents of the larger ducts. Many ducts were distended with retained secretion and cellular debris and some contained very large cells morphologically similar to colostrum corpuscles (Fig. 23). In many sections, dense lymphocytic foci within lobules were a prominent feature of this stage of the disease (Fig. 24). In one animal inoculated late in lactation and in which there was evidence of involutionary change in the non-inoculated side of the udder, a variation of this stage of the disease was seen; this is depicted in Figure 25, which shows the macrophage-fibroblast reaction together with marked evidence of involution, this degree of involution being

considerably greater than in the non-inoculated side of the same udder.

The stage of the disease just described, showing stasis of secretion and intense interacinar cellularity, was obvious in animals killed nine days after inoculation and corresponded with the clinical period of maximum swelling and tensity of the udder.

In subsequent stages of the disease, involutionary changes in the lobules were well marked. The macrophage-fibroblast reaction gave way to one that was predominantly fibroblastic, the acinar structure was completely lost, but terminal ductules, many containing numerous neutrophils, were prominent (Fig. 26). Cocci were found, only with difficulty, in pockets in the larger ducts. It is possible that examination of sections showing this stage of the disease may have prompted Morrill to comment as follows, ". . . in numerous areas already greatly altered by chronic productive [meaning fibrosis?] changes, myriads of polymorphs are seen infiltrating the connective tissue and exuding into what alveoli are left. Whether this phenomenon is an expression of a change in virulence or numbers of an injurious agent already present, or of a secondary invasion of the area by another agent is not determined." Neither explanation is entirely acceptable; another possibility seems to be that this change is merely part of the process of involution, complicated and almost hidden by the co-existing cellular reaction.

Following this stage in the disease process, some lobules showed excessive fibrosis (Fig. 27), others a much closer approach to normal involution (Fig. 28) and the size of the lobules themselves was continually reduced.

Examination of udders in the end stages of the disease, when lactation had virtually ceased and clinical atrophy was very marked, showed the lobules to be in conditions that varied from almost normal involution (Fig. 29) to almost complete fibrosis (Fig. 30); intermediate stages showing fibrosis but also some attempt at involution (Fig. 31) were numerous. In some animals, dense paraductal lymphocytic foci (Fig. 32) were a marked feature of the latest stage of the disease. As had been reported previously, the viable count of streptococci per ml. of secretion from such an udder was always small; histologically organisms were hard to find and appeared to be confined to the few remaining patent ducts. The time taken for a lobule to be reduced to this functionless state probably varied considerably, but apparently could be remarkably short. Figure 28 is from a goat killed on the ninth day after inoculation, Figures 27, 29 and 30 are from an animal killed on the 36th day and Figures 31 and 32 are from an animal killed on the 82nd day.

McFarlane and Rennie have given a classification of changes in the bovine mammary gland in which is included what is termed "productive change in involuted tissue." This change is described

as representing "the end result of a progressive mastitis starting as a typical exudative inflammation in lactating tissue, followed by progressive and continuous fibroblastic activity until the lactating tissue is forced by lack of space as well as by the continuance of the inflammatory process to a stage of full involution." I cannot agree that lack of space is responsible for the regressive changes that accompany an active inflammatory reaction in the udder. I believe that involution is a defence reaction on the part of the organ and an integral part of the histological picture of the disease process.

A different type of pathological change that involved extensive areas of lobular tissue in some goat udders and consisted of fibrosis without an accompanying macrophage reaction, will be commented on later as it was apparently initiated by a special type of reaction that occurred in some ducts.

Changes in ducts

Two different types of change were noted in the ducts of animals that gave a marked reaction to inoculation of living streptococci. The first of these was found mainly in the larger ducts, and the second in the smaller ducts and ductules.

Changes in the larger ducts. These changes were seen in some ducts six hours after inoculation and consisted of infiltration beneath the epithelium of the duct wall with macrophages and a smaller number of neutrophils (Fig. 33). This infiltration increased rapidly in intensity, the surface epithelium showed hyperplasia and fibroblasts were added to the infiltrating cells (Fig. 34); the ducts themselves were widely dilated. Within the ducts, streptococci were numerous in regions of marked infiltration and were frequently found deep in the layer of hyperplastic epithelial cells. The fibroblast reaction deep to the surface epithelium was then found to increase in intensity, so that papilliform projections were thrown out into the duct lumen (Fig. 35) and the surface epithelium lost its irregular hyperplastic appearance and became stratified; streptococci were much less easily found at this time. Later, the surface epithelium changed to a stratified, squamous type and in some cases was even cornified (Fig. 36). Figures 35 and 36 are from an animal killed 27 days after inoculation and Figure 37 gives the macroscopic appearance of this udder, with papillary projections showing in the larger ducts. Similar papillary projections were noted in the bovine udder by Nieberle and Cohrs (1931), and Scholl (1946) has described the cornification of duct epithelium in some cases of bovine mastitis.

These changes within the larger ducts were present during the stages of the disease characterised by progressive swelling and induration. As the udder began to involute and the process of fibrosis to become dominant (characterised clinically by progressive reduction in size) the stratified epithelial lining of the ducts was lost by desquamation of the surface layers of cells until finally the duct lumen was quite narrow and the walls were lined with

epithelium similar to that noted in the normal involuted gland (see Fig. 38, 71 days after inoculation).

Changes in the smaller ducts: This type of abnormality began as a small invagination of epithelium into a duct that was invariably dilated and contained a certain amount of cell debris in which streptococci could usually be found (Fig. 39). This invagination increased rapidly in size (Figs. 40 and 41), and there was usually evidence of hyperplasia of its epithelial covering and sometimes of hyperplasia of the epithelial lining of the duct (Fig. 41). The increase in size continued, the epithelial covering breaking down in places (Fig. 42) until a large body of granulation tissue, with a greater or lesser amount of epithelium, now protruded into a vastly dilated duct (Fig. 43); sometimes there was more than one point of origin through the duct wall (Fig. 44). A variation in this type of ingrowth sometimes found is shown in Figure 45, where about two-thirds of the duct wall have been invaginated to give a mass of granulation tissue that almost fills the duct. If the plane of section did not pass through the point of origin of these ingrowths, they had the appearance of being loose within the duct (Fig. 46). In some instances, this development of granulation tissue within a duct, originating as an invagination through the wall, almost completely filled the duct lumen (Fig. 47).

This type of intraductal invagination in the mammary gland is not peculiar to the goat. It has been observed (Fig. 48) in inflammatory conditions of the human breast (Willis, 1950), and I have seen it in the bitch (Fig. 49). It does not appear to have been directly described in the cow, although Spencer and McNutt (1950) have depicted in Figure eight of their paper what is termed "an organized clot in a collecting duct," a lesion that bears a striking resemblance to that described above. Figure four in the paper on bovine mastitis by Chu (1949) is very like Figure 47 presented here. McFarlane and Rennie spoke of "glands showing obliterative duct lesions," without further description of these lesions, and it seems probable that they were referring to ingrowths similar to those described above.

It has been said that in some instances the final result of these fibrous ingrowths was blockage of the duct. In other cases, the progress of the lesion was entirely different. The duct continued to dilate and the mass of granulation tissue within it to extend, but towards one surface of the granulation mass, epithelial elements appeared and became arranged into a stratified, pavement covering, their free surface being towards the lumen of the duct and such debris (almost always containing streptococci) as remained there (Fig. 50). In some instances, the dilation of the duct was so great and the mass of granulation tissue within it so large that the intraductal origin of the lesion was not easily detected; at some point, however, duct epithelium separated from granulation tissue could usually be found (Fig. 51). The next stage in the process was fusion

between the intraductal granulation tissue and the duct wall, the duct epthelium being lost, so that were it not for the epithelium at the free surface of the granulation tissue, the lesion could well be mistaken for an abscess. The resemblance to an abscess was heightened by the development of dense fibrous tissue round the line of what had originally been the duct wall. The very considerable size of some of these lesions and the rapidity of their development, may be judged from Figure 52, that shows the macroscopic appearance of the udder of an animal destroyed nine days after inoculation; the pale rounded areas are lesions of this type.

During development of the lesion intraductally the adjacent lobular tissue had been undergoing profound changes. The connective tissue reaction, initiated in the periductal tissue and continued intraductally as a rapid proliferation of granulation tissue, had progressed extraductally and spread outwards to involve a very considerable area of lobular tissue. An area involved in spreading fibrosis of this type is shown in Figure 53 (from an animal killed nine days after inoculation), and is explained diagrammatically in Figure 53*a*. Point A is the duct lumen containing cell debris (as shown in Fig. 50); B is stratified, pavement epithelium (as shown in Fig. 50) forming an incomplete ring of variable thickness along the free surface of the duct; C is granulation tissue (as shown in Fig. 50), marking the original limit of the duct wall and shading into the surrounding dense connective tissue; D and D′ define the aproximate limits of development of dense fibrous tissue in which some lobules have been almost obliterated (Fig. 54); E is lobular tissue still recognisable as such, but buried in fibrous tissue (Fig. 55); F is lobular tissue involved to a lesser degree in the spreading fibrosis (Fig. 56); G is lobular tissue involved in the process only to a relatively small extent (Fig. 57). In none of these areas was there evidence of a macrophage reaction preceding the fibrosis and the change, therefore, appeared to differ fundamentally from the more usual fibrosis of lobular tissue already described. Evidence of involutionary change was also absent. The finding in a section of the type of fibrosis shown in Figure 56 indicated the presence in the vicinity of an intraductal lesion of the type described, and examination of further sections from the same block of tissue usually showed this to be the case.

These intraductal lesions were most prominent during the clinical period of maximum swelling and tensity of the udder. As the disease developed, they gradually lost their well-defined structure. The pavement epithelium shed its surface layers (the duct remaining patent) and the surrounding tissue contracted to a dense fibrous mass. A granulation tissue ingrowth undergoing this type of change is shown in Figure 58; the cells in the duct are shed-off epithelial cells. The final result was a contracted mass of fibrous tissue surrounding a narrow duct lined by epithelium of the type seen in involuted tissue.

DISCUSSION

Spencer and McNutt have drawn attention to the confusion that now exists in the terminology used by the various authors who have described histopathological changes in bovine mastitis. Most authors have tried to classify their findings, or to summarise them in short phrases such as, "acute exudative mastitis," or "chronic productive mastitis." Unfortunately these classifications are mainly subjective and adequate definition of the phrases in question has rarely been given.

Simplification of a complex pathological picture is justifiable in the cause of better understanding. But classification of the findings in one disease under a number of ill-defined and apparently unconnected headings does not necessarily constitute successful simplification. In this study of goat udders inoculated with *Str. agalactiae,* all the classes of change described by authors who have studied bovine streptococcal mastitis have been encountered, and very frequently several of these changes have been visible in the same microscopic section. Can these changes, therefore, be separated from each other? Surely they are interdependent and are manifestations of only *one* disease process? At best, their separation from each other forms only a classification of individual microscopic fields.

The use of such terms as "catarrhal," "dormant," "extinct" and so on is to be deprecated until each has a precise meaning. For the present, a simple description of cellular changes seems preferable to the use of terms that are too vague to convey an adequate picture of the pathological process under discussion.

SUMMARY

A histological examination was made of the udders of 39 goats inoculated into the teat canal of one side of the udder with heat-killed or living *Str. agalactiae* strain S13.

The reaction to heat-killed organisms was a slight, patchy and transient migration of neutrophils into secreting acini.

Histological findings in animals inoculated with living streptococci varied according to the type of clinical reaction (minimal, intermediate or marked as described previously).

Minimal reactions were characterised by an early migration of neutrophils into the secreting acini, followed by a slight macrophage-fibroblast reaction in the interacinar tissue. These changes were regarded as non-specific. There was no evidence of penetration of streptococci through the duct walls.

Marked reactions were characterised by an early migration of neutrophils into secreting acini. The essential feature in all marked reactions was penetration of streptococci through the duct and acinar epithelium to the lymphatic channels. This penetration was brief and the organisms were rapidly destroyed, but it initiated a marked macrophage-fibroblast reaction. There was extensive damage to secreting tissue and very early evidence of

involution. The disease progressed rapidly and ended in involution together with a greater or lesser degree of fibrosis. Some of the larger ducts showed papilliform outgrowths and development of a stratified, pavement epithelial lining during the height of the disease. In some smaller ducts ingrowths of granulation tissue were found developing in some cases to a considerable size and involving surrounding lobular tissue in extensive fibrosis.

Cases of intermediate reaction to inoculation showed the same changes as in the marked reaction cases, but with much less extensive involvement of udder tissue.

Penetration of the epithelium of the duct system by the invading organism was essential to the development of the classical type of reaction that closely resembled bovine streptococcal mastitis. Its occurrence coincided in many cases with a rise in body temperature of the animal and with a high viable count of streptococci per ml. of milk.

There was no essential difference between histological findings in goats infected experimentally and those reported in the literature in natural cases of bovine streptococcal mastitis.

ACKNOWLEDGMENTS

Dr. E. K. Dawson, of the Royal College of Physicians' Laboratory, Edinburgh (now, University Pathology Department, Forrest Road Laboratory), to whom I am indebted for reading and discussing this paper and for helpful criticism, has found that the changes seen in infective inflammatory conditions of the human breast are very similar to those described here in goats inoculated with streptococci.

I would like to thank my Director, Dr. W. S. Gordon, C.B.E., for his constant interest in this work and for providing the facilities for its prosecution. My special thanks are due to my colleague Dr. H. H. Holman for encouragement and constructive criticism, and to Mr. F. H. Summerfield, Senior Technician of this Laboratory, for the patience and the skill with which he prepared the illustrations.

Reproduction of the illustrations was made possible by a special grant from the Agricultural Research Council.

REFERENCES

Andberg, W. G., and Weirether, F. J. (1943). *Amer. J. vet. Res.*, **4,** 134; (1944). *Ibid.*, **5,** 6.

Bryan, C. S. (1946). *In* Little and Plastridge's *Bovine Mastitis.* New York.

Chu, S. J. (1949). *J. comp. Path.*, **59,** 81.

Dawson, E. K. (1935). *Edin. med. J.*, **42,** 569.

Holman, H. H., Pattison, I. H., and Taylor, J. I. (1950). *J. comp. Path.*, **60,** 100.

M'Fadyean, J. (1930). *Ibid.*, **43,** 291.

McFarlane, D., and Rennie, J. C. (1950). *Aust. vet. J.*, **26,** 53.

Morrill, C. C. (1938). *Cornell Vet.*, **28,** 196.

Nieberle, K., and Cohrs, P. (1931). *Lehrbuch der Speziellen Pathologischen Anatomie der Haustiere.* Jena.

Pattison, I. H., Taylor, J. I., and Holman, H. H. (1950). *J. comp. Path.*, **60,** 71.
Pattison, I. H., and Holman, H. H. (1951). *Ibid.*, **61,** 26.
Richardson, K. C. (1947). *Brit. med. Bull.*, **5,** 123.
Sholl, L. B. (1946). *In* Little and Plastridge's *Bovine Mastitis.* New York.
Shennan, T. (1929). *J. Path. Bact.*, **32,** 164.
Spencer, G. R., and McNutt, S. H. (1950). *Amer. J. vet Res.*, **11,** 188.
Taylor, J. I., Holman, H. H., and Pattison, I. H. (1950). *J. comp. Path.*, **60,** 92.
Turner, C. W., and Reineke, E. P. (1936). *Res. Bull. Mo. agric. Exp. Sta.*, No. 235.
Turner, C. W. (1939). *The Comparative Anatomy of the Mammary Glands.* Columbia Mo., Univ. Coop. Store.
Willis, R. A. (1950). Personal communication.

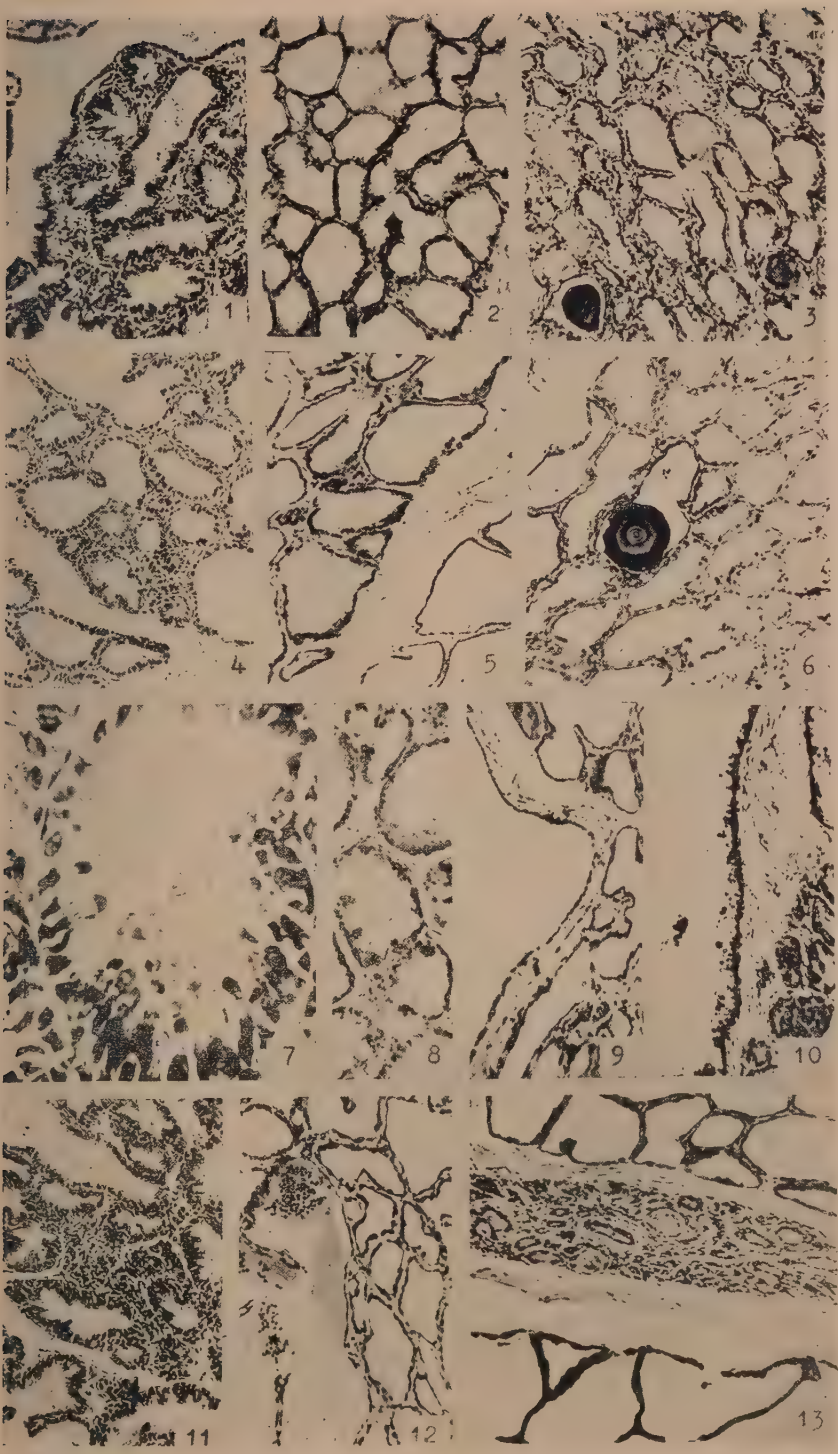
Work consulted

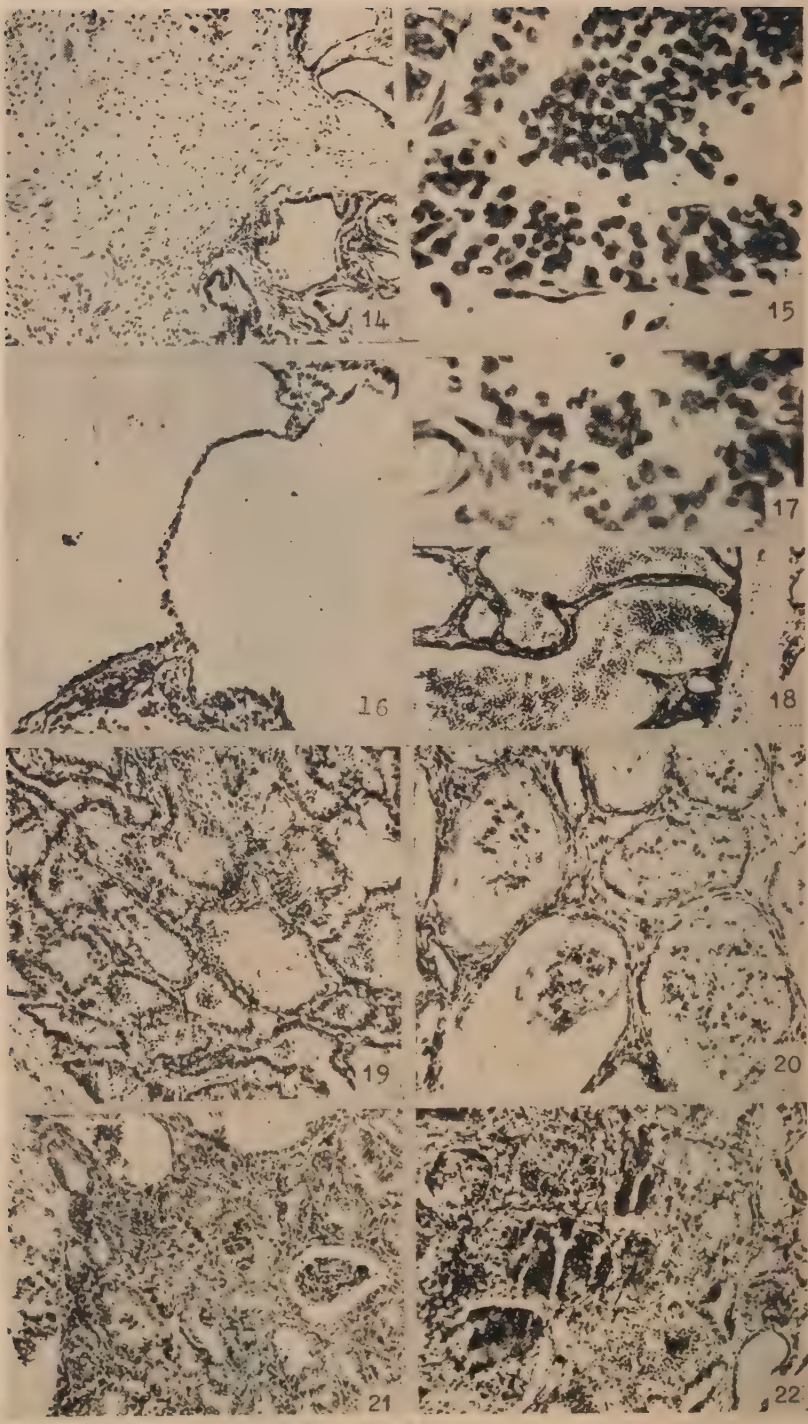
Cheatle, G. L., and Cutler, M. (1931). *Tumours of the Breast.* London.

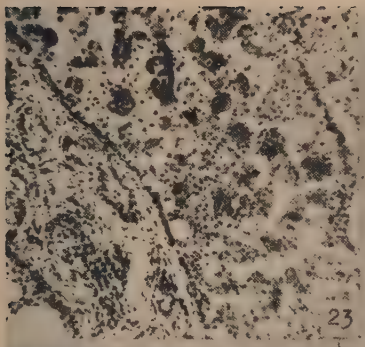
[*Received for publication, November 15th,* 1950.]

LEGENDS TO ILLUSTRATIONS

FIG. 1. Non-inoculated udder; early lactation. H. & E. × about 75.
FIG. 2. Non-inoculated udder; mid-lactation. H. & E. × about 75.
FIG. 3. Non-inoculated udder; late lactation; corpora amylacea. H. & E. × about 75.
FIG. 4. Non-inoculated udder; gland milked out before death. H. & E. × about 75.
FIG. 5. Non-inoculated udder; gland not milked out before death. H. & E. × about 75.
FIG. 6. Non-inoculated udder; corpora amylacea. H. & E. × about 75.
FIG. 7. Non-inoculated udder; secreting acinar epithelium. H. & E. × about 400.
FIG. 8. Non-inoculated udder; acinar epithelium showing "bunch-of-grapes" formation. H. & E. × about 75.
FIG. 9. Non-inoculated udder; non-secretory epithelium lining a large duct. H. & E. × about 75.
FIG. 10. Non-inoculated udder; secretory epithelium lining a large duct. H. & E. × about 75.
FIG. 11. Non-inoculated udder; small focus of lymphocytes. H. & E. × about 75.
FIG. 12. Non-inoculated udder; ductule containing eosinophilic debris and a few neutrophils. H. & E. × about 75.
FIG. 13. Non-inoculated udder; pre-functional lobule. H. & E. × about 75.
FIG. 14. Extensive exudation of neutrophils in interlobular tissue (6 hours after inoculation). H. & E. × about 75.
FIG. 15. Neutrophils passing through the wall of an acinus (6 hours after inoculation). H. & E. × about 400.
FIG. 16. Greatly dilated lymphatic vessel (showing valve) with cellular infiltration in the wall (9 days after inoculation). H. & E. × about 75.
FIG. 17. Neutrophils in the medullary region of the supramammary lymphatic gland (6 hours after inoculation). H. & E. × about 400.
FIG. 18. Small focus of migrating neutrophils (36 days after inoculation). H. & E. × about 75.
FIG. 19. Developing macrophage-fibroblast reaction (9 days after inoculation). H. & E. × about 75.
FIG. 20. Stringy eosinophilic material and cellular debris in acini (4 days after inoculation). H. & E. × about 75.
FIG. 21. Early obliteration of acinar structure (4 days after inoculation). H. & E. × about 75.
FIG. 22. Retained secretion; markedly increased interacinar cellularity; early evidence of involution (9 days after inoculation). H. & E. × about 75.
FIG. 23. Duct distended with retained secretion; some cells similar to colostrum corpuscles (9 days after inoculation). H. & E. × about 75.
FIG. 24. Dense focus of lymphocytes (9 days after inoculation). H. & E. × about 75.
FIG. 25. Macrophage-fibroblast reaction in partially involuted tissue (9 days after inoculation). H. & E. × about 75.
FIG. 26. Terminal ductules containing numerous neutrophils; prominent fibroblast reaction (36 days after inoculation). H. & E. × about 75.
FIG. 27. Progressive fibrosis (36 days after inoculation). H. & E. about 75.
FIG. 28. A little fibrosis together with involution (9 days after inoculation). H. & E. × about 75.
FIG. 29. Almost normal involution (36 days after inoculation). H. & E. × about 75.
FIG. 30. Almost complete fibrosis (36 days after inoculation). H. & E. × about 75.
FIG. 31. Fibrosis plus some attempt at involution (82 days after inoculation). H & E. × about 75.
FIG. 32. Paraductal lymphocytic focus (82 days after inoculation). H. & E. × about 75.

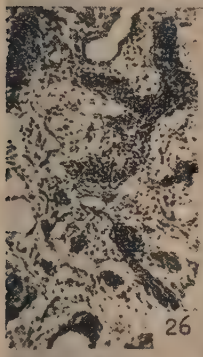

1
2
3
4
5
6
7
8
9
10
11
12
13

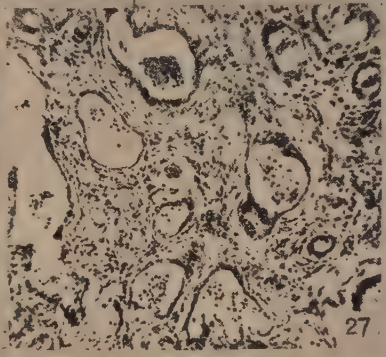

14
15
16
17
18
19
20
21
22

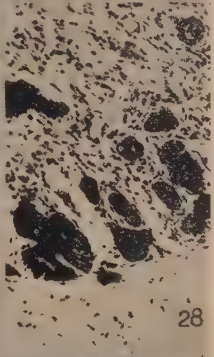

23

24

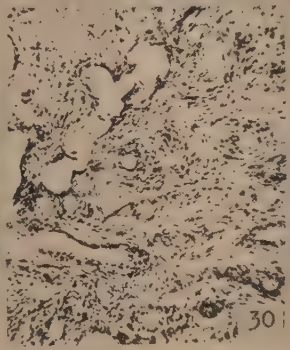
25

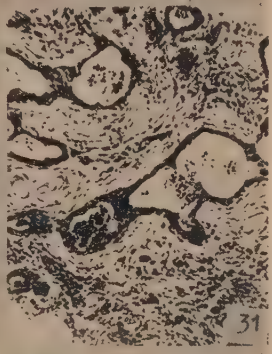

26

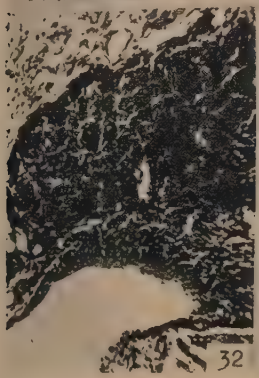

27

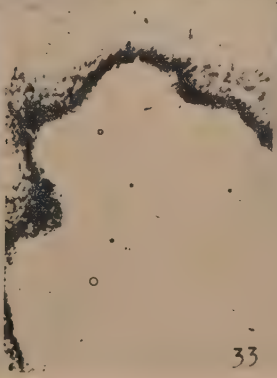

28

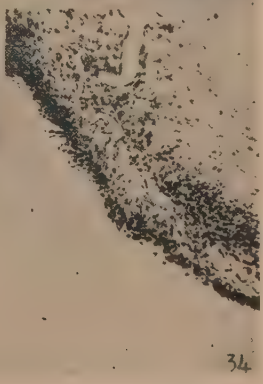
29

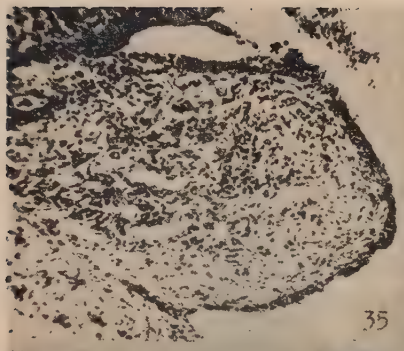

30

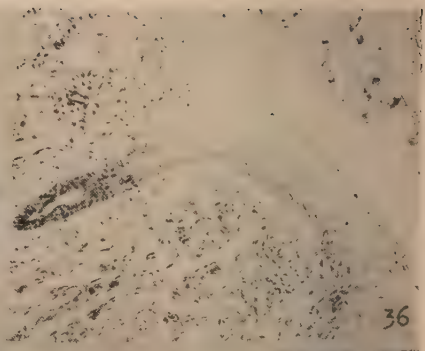

31


32

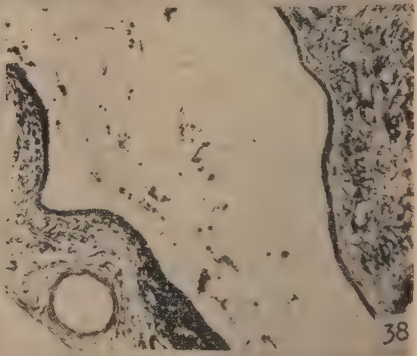

33

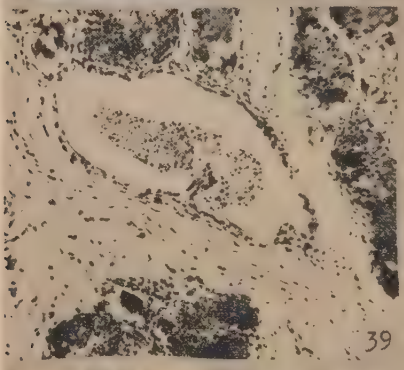

34

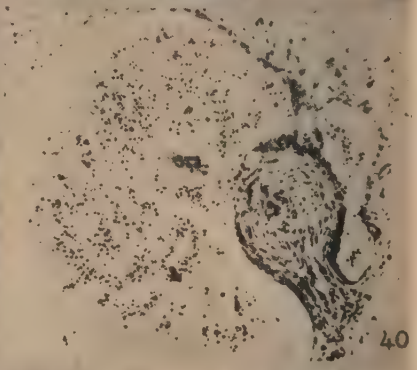

35

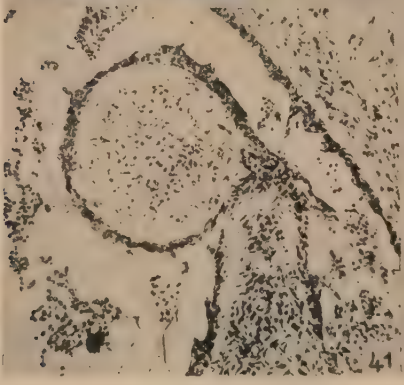

36

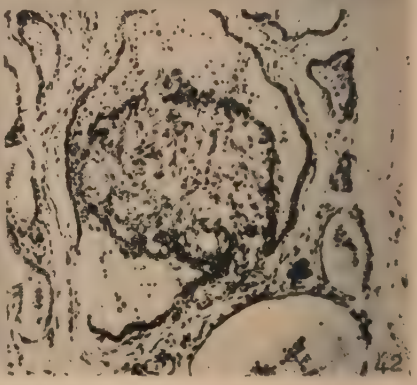
37

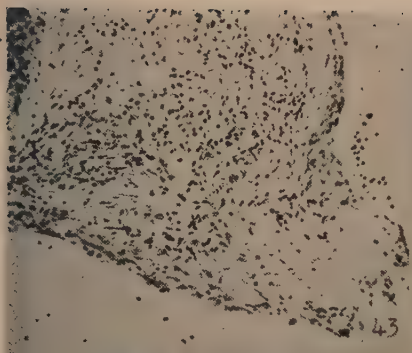

38

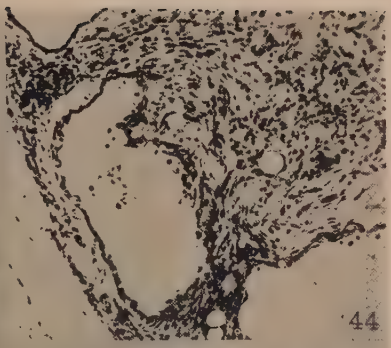

39


40

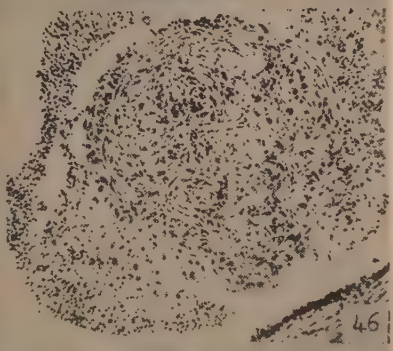

41

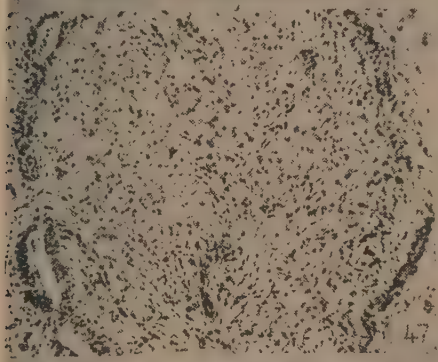

42


43


44

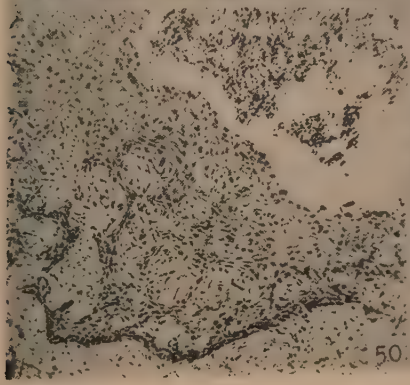
45


46


47

48

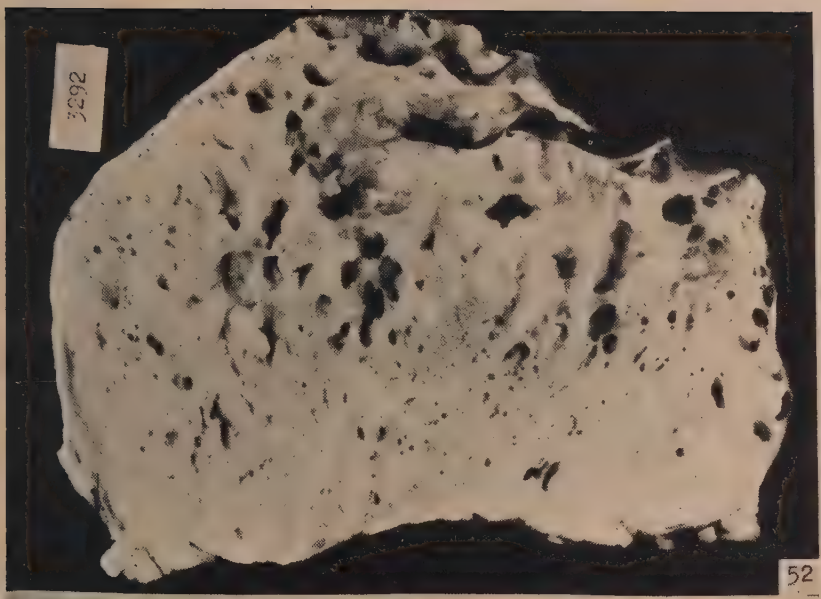
49


50

51

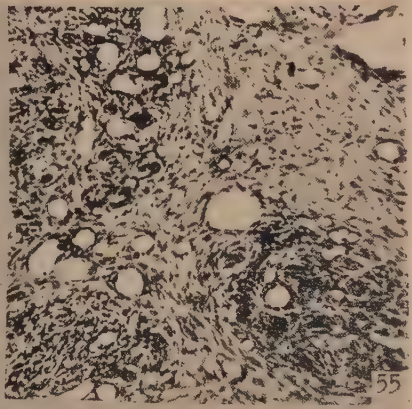
53

53a

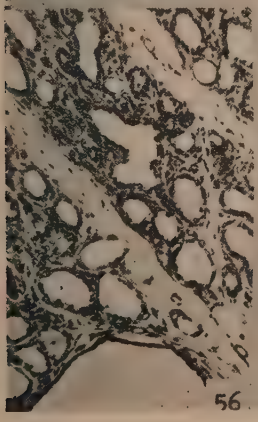


52

54

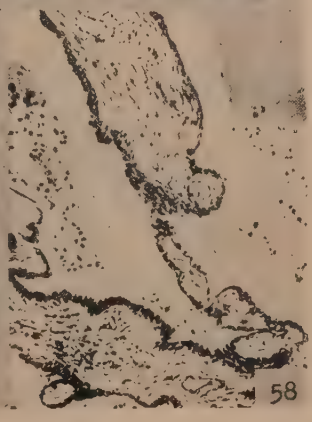


55



56

57



58

FIG. 33. Large duct; periductal cell infiltration (9 days after inoculation). H. & E. × about 75.

FIG. 34. Large duct; epithelial hyperplasia (9 days after inoculation). H. & E. × about 75.

FIG. 35. Large duct; papilliform projections (27 days after inoculation). H. & E. × about 75.

FIG. 36. Large duct; stratified, cornified epithelium (27 days after inoculation). H. & E. × about 75.

FIG. 37. Udder of goat killed 27 days after inoculation showing papilliform projections into large ducts. Slightly reduced from natural size.

FIG. 38. Duct epithelium in a late stage of the disease (71 days after inoculation). H. & E. × about 75.

FIG. 39. Earliest stage of invagination of epithelium into a small duct (9 days after inoculation). H. & E. × about 75.

FIG. 40. Invagination into a small duct; epithelial covering almost intact (9 days after inoculation). H. & E. × about 75.

FIG. 41. Invagination; marked epithelial hyperplasia (27 days after inoculation). H. & E. × about 75.

FIG. 42. Invagination; epithelial covering breaking down (9 days after inoculation). H. & E. × about 75.

FIG. 43. Invagination now a large body of granulation tissue in a greatly dilated duct (9 days after inoculation). H. & E. × about 75.

FIG. 44. An invagination with two points of origin (9 days after inoculation). H. & E. × about 75.

FIG. 45. A granulation tissue body originating from the greater part of the duct wall (9 days after inoculation). H. & E. × about 75.

FIG. 46. The plane of section makes this granulation tissue body appear loose within the duct (9 days after inoculation). H. & E. × about 75.

FIG. 47. Granulation tissue body almost blocking a duct (27 days after inoculation). H. & E. × about 75.

FIG. 48. Human breast; small invaginations of the duct wall (slide obtained through the kindness of Professor R. A. Willis). H. & E. × about 75.

FIG. 49. Mammary gland of bitch; growth of granulation tissue into a duct. H. & E. × about 75.

FIG. 50. Stratified epithelium at the free surface of a granulation tissue ingrowth; see also Figs. 53 and 53a (9 days after inoculation). H. & E. × about 75.

FIG. 51. Mass of granulation tissue lying within a greatly dilated duct (9 days after inoculation). H. & E. × about 75.

FIG. 52. Udder of animal killed 9 days after inoculation showing numerous lesions such as that in Figs. 53 and 53a. Slightly reduced from natural size.

FIG. 53. Lesion developed from an invagination into a small duct; see Fig. 53a and text (9 days after inoculation). H. & E. × about 20.

FIG. 53a. The lesion shown in Fig. 53.

A—Cell debris.

B—Stratified epithelium (see Fig. 50).

C—Granulation tissue marking the original limit of the duct wall.

D and D^1—The approximate limits of development of dense fibrous tissue (see Figs. 54 and 55).

E—Lobular tissue buried in fibrous tissue (Fig. 55).

F—Lobular tissue less involved in the spreading fibrosis (see Fig. 56).

G—Lobular tissue only slightly involved in the spreading fibrosis (see Fig. 57).

FIG. 54. Points D—D^1 in Fig. 53a. H. & E. × about 75.

FIG. 55. Point E in Fig. 53a. H. & E. × about 75.

FIG. 56. Point F in Fig. 53a. H. & E. × about 75.

FIG. 57. Point G in Fig. 53a. H. &. E. about 75.

FIG. 58. Breakdown of a granulation tissue ingrowth; the cells in the duct are epithelial (71 days after inoculation). H. & E. × about 75.

THE EXPERIMENTAL PRODUCTION OF BRACKEN "POISONING" IN SHEEP

By

F. E. Moon and M. A. Raafat.

The Edinburgh and East of Scotland College of Agriculture.

INTRODUCTION

Since the first recorded case of bracken poisoning in cattle there have been numerous outbreaks of the condition, although their number has varied considerably from year to year. Although the condition has been attributed by some observers to causes other than the consumption of bracken (*Pteris aquilina*) the toxic nature of the plant is now fairly generally accepted, even if in its effects the exact cause of its toxicity is still obscure. An anti-thiamin activity of bracken has been demonstrated by several workers and the significance of this in the production of toxic effects in rats and horses has recently received considerable attention (Weswig, Freed and Haag, 1946; Haag, Weswig and Freed, 1947; Haag and Weswig, 1948; Thomas and Walker, 1949; Evans and Evans, 1949; Roberts, Evans and Evans, 1949; Evans, Jones and Evans, 1950). In the production of bracken poisoning in ruminants, however, the antithiamin activity of the plant may be of much less significance. The symptoms of the condition in ruminants are quite different from those of bracken staggers in the horse and from the effects produced in rats, and the post-mortem picture is also entirely different. In the ruminant animal the symptoms of bracken poisoning have been fully described by Stockman (1917) and recently summarised by Stamp (1947). There is invariably a marked rise in body temperature and the post-mortem findings are characterised by widespread haemorrhages of the gastro-intestinal tract, particularly of the walls of the abomasum and small intestine.

The condition has been produced experimentally in cattle by several workers including Stockman (1917, 1922) and Shearer (1945), although attempts to induce it have not always been successful. In sheep, the natural occurrence of the condition would appear to be very rare, if it exists at all in this country, and there does not appear to be any record of its production experimentally. The possible poisoning of sheep in New Zealand by fern was reported by Hickman (1910), but the evidence was somewhat circumstantial and the post-mortem findings described by him were not characteristic of bracken poisoning. Fletcher (1944) has reported the case of a black-face ewe found in Scotland with post-mortem lesions of bracken poisoning, although the evidence in that case was incomplete, and since the lesions of enterotoxaemia in sheep may be confused with those of bracken poisoning it is

clear that further evidence is necessary to show that the plant can produce in sheep the same symptoms as in cattle.

The elucidation of the factors responsible for bracken poisoning in ruminants is of considerable economic importance since the control and prevention of this disease is necessary if full use is to be made of the grazing potentialities of the hill land of Britain. As research on this problem would be much easier and less costly if sheep could be used instead of cattle, attempts have been made to produce bracken poisoning experimentally in sheep. Half-bred and Oxford cross wethers, varying in age from one to two years, were used and experiments were carried out with (*a*) fresh bracken, (*b*) artificially dried bracken and (*c*) cubed bracken meal. In all cases bracken material formed the sole diet. As sheep are more selective than cattle in their feeding habits it was found necessary to exclude all other foods from the ration in order to secure an adequate consumption of bracken. It was found that provided sufficient quantities of bracken were eaten, the sheep were able to make sustained live weight increases, confirming the by no means negligible nutritive value of bracken reported in a previous paper (Moon and Pal, 1949).

THE FEEDING OF FRESH BRACKEN

Attempts were made to feed four sheep, C, D, E and F, with bracken collected fresh each morning from the Pentland hills. The bracken was chopped in a power-driven chaff cutter and well

TABLE I

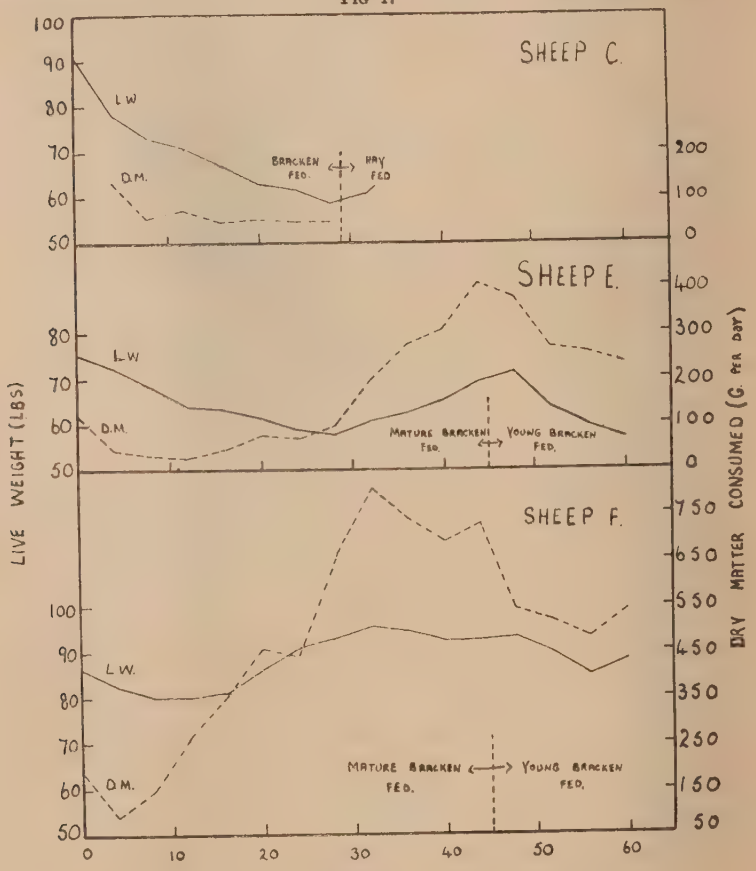
FRESH BRACKEN FEEDING EXPERIMENTS.

Sheep	*Duration of Experiment*	*No. of days*	*Dry weight of bracken consumed*		*Result*
			Total (Kg.)	*Av. per day (g.)*	
C	June 18—July 16	29	1·76	61	Survived
D	Aug. 18—Aug. 25	8	0·56	70	Survived
E	Aug. 18—Oct. 20	64	11·13	174	Died without symptoms of bracken poisoning.
F	Aug. 18—Oct. 22	66	33·29	504	Died with bracken poisoning.

mixed before feeding, samples being taken for dry matter determination. Uneaten residues were collected for weighing and the dry matter contents of the residues were also ascertained. The consumption of bracken dry matter is summarised in Table I and the influence on the liveweights of the sheep is shown in Figure I.

Sheep C was fed bracken for 29 days in June and July, 1949, but the amount of dry matter eaten was very small, averaging only 61 g. per day. The weight of this animal fell from 91lb. at the commencement of the experiment to 62 lb. at the end, a loss of

FIG 1.



LIVE WEIGHTS AND FOOD CONSUMPTION IN FRESH BRACKEN FEEDING EXPERIMENTS.

1 lb. per day. The experiment with this sheep was then abandoned as the food consumption was quite inadequate. No symptoms of bracken poisoning were produced.

Sheep D was offered fresh bracken for eight days in August, 1949, but proved an even more unsatisfactory experimental animal. On the first day it ate 295 g. bracken dry matter, but

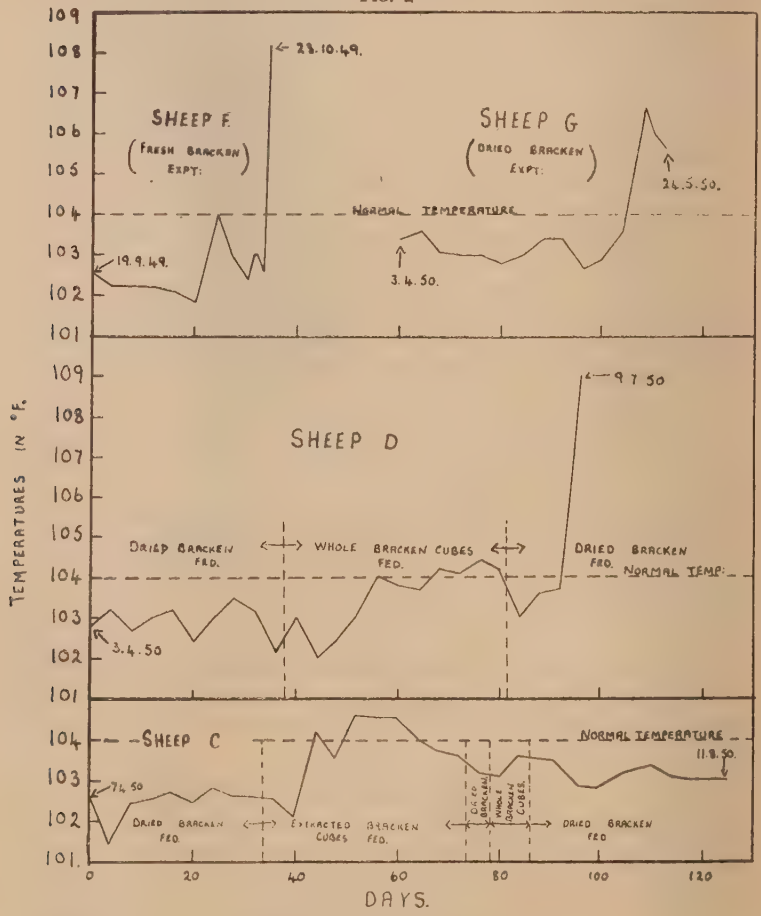
thereafter it almost completely refused to eat, consuming an average of only 38 g. dry matter per day. As it became very weak the experiment was abandoned.

Sheep E was fed bracken for 64 days from August to October, 1949. During the first part of this period (August 18th to October 8th) mature bracken was fed but thereafter a young second growth of bracken was used from an area cut in the previous June and July. This sheep consumed an average of 174 g. bracken dry matter per day and at the commencement of the experiment when consumption was only in the region of 50 g. dry matter daily there was a marked loss in body weight. Subsequently, there was an increase in consumption and a similar recovery in body weight (Figure I). Following the change-over to young second-growth bracken in October, there was again a rapid decline in the animal's weight until by the end of the experiment it had fallen from the initial value of 75·5 lb. to 56·5 lb. and death occurred. This sheep did not, however, show any symptoms typical of bracken poisoning; there was no abnormality of any sort until October 21st when it was found in a comatose condition. The temperature remained subnormal; on October 13th it had been 101° F. and on October 21st it was only 95° F. There was no blood in the faeces and no other indications of haemorrhage. Despite the administration of thiamin this animal died the same day and post-mortem examination showed a complete absence of any haemorrhage or congestion in the intestinal tract. This was clearly not a case of typical bracken poisoning and although the symptoms were suggestive of thiamin deficiency the failure to respond to Vitamin B_1 injection did not support this theory.

Sheep F consumed fresh bracken at the same time as Sheep E, but ate considerably larger quantities. At the commencement of the experiment when food consumption was low there was an initial loss of about 7 lb. live weight but after a few days there was a recovery of appetite followed by an increase in live weight until the young second growth of bracken was fed on October 9th. During this latter period consumption and live weight again declined a little. Throughout the bracken feeding the body temperature, shown in Figure II, remained between 101·8 and 102·6° F. until October 12th, when it rose to 104° F. The animal was grinding its teeth and was off its feed. On October 13th the temperature was still 104° F. and the faeces became very soft. Between October 13th and 22nd the temperature fluctuated from 102·4° F. to 104·0° F. and on the latter date the faeces were very soft with considerable quantities of mucus and many blood clots. On October 23rd the temperature (Figure II) rose sharply to 108° F. and the faeces were very soft and foetid, with many blood clots. Vitamin B_1 injection was again tried without avail, and the animal died on October 23rd. The symptoms observed and the post-mortem findings were typical of bracken poisoning in cattle,

there being many internal haemorrhages and blood clots. There were multiple petechial haemorrhages of the abomasum wall and extensive areas of congestion on the walls of both the small and large intestines. Haemorrhages were also found in the kidney, spleen and heart and the lungs had a purple mottled appearance.

Fig. 2



RECTAL TEMPERATURES
of
EXPERIMENTAL ANIMALS.

During the long period August 18th to October 8th (52 days) when Sheep E and F were fed on fully mature bracken, no effects other than low temperatures were observed; the faeces were normal in appearance and the sheep showed increases in live weight. When, however, the animals were fed on young bracken from

October 9th, both became ill and died, and in the case of Sheep F in which symptoms developed in the very short period of about 14 days, the symptoms closely resembled bracken poisoning of cattle. A similar result occurred with a bullock fed on the same bracken at the same time. The feeding of mature bracken from August 18th to October 8th had no adverse effect but the animal died on October 24th after only a relatively short period of feeding on young bracken. There was apparently a great difference in toxicity between the two types of bracken.

THE FEEDING OF ARTIFICIALLY DRIED BRACKEN

Since the maturity of bracken appeared to influence its toxicity and investigational work on this problem had been hampered by the relatively short period of time in each year when suitable material was available for feeding, the possibility of conserving bracken by artificial drying was investigated in order to make experimental work possible during the winter and spring months. For this purpose young green bracken was cut on June 30th, 1949 and dehydrated in a commercial, oil-fired continuous belt drier, operating with an inlet air temperature of about 300° F., the exhaust temperature being in the range 130 to 150° F. The artificially dried bracken was stored during the summer and winter. In March, 1950 it was fed to Sheep C and D, used in the previous experiment, and a new sheep G. Table II shows the consumption of dried bracken by these sheep and Figure III the effect on body weights. It will be noted that with sheep C and D the feeding of dried bracken was not continuous. Cubed bracken

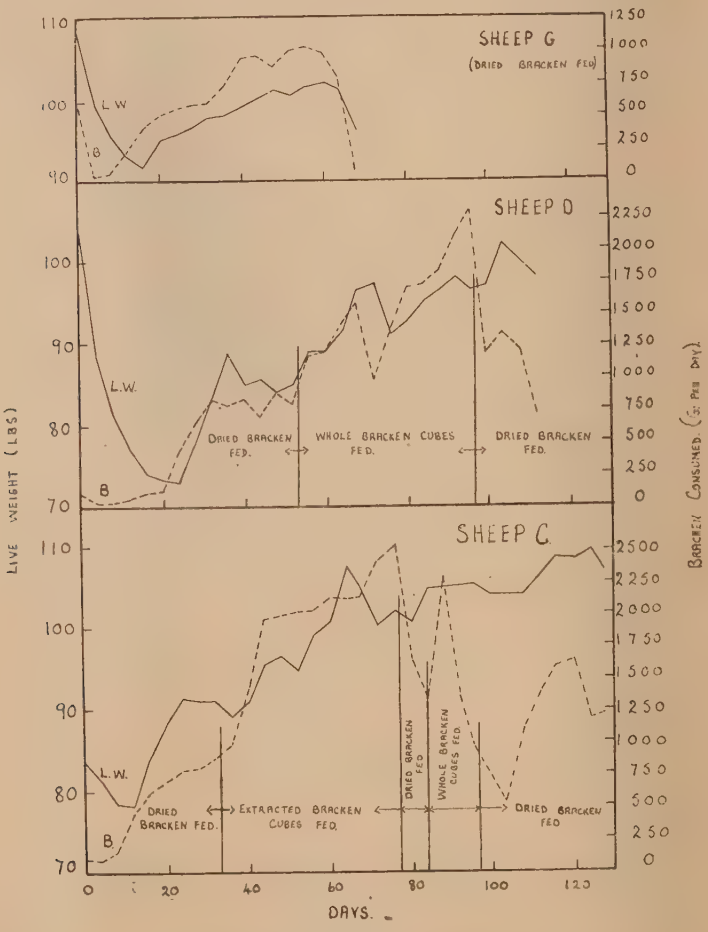
TABLE II

DRIED BRACKEN FEEDING EXPERIMENTS.

Sheep	*Duration of Experiment*	*No. of days*	*Dried bracken consumed*		*Result*
			Total (Kg.)	*Average per day (g.)*	
C	Apr. 3—May 10	38	20·52	540	Survived
C	June 24–28	5	5·07	1014	Survived
C	July 9—Aug. 10	33	38·76	1175	Died with bracken poisoning 7 days after termination of bracken feeding.
	TOTAL ...	76	64·35	847	
D	Mar. 16—May 10	56	25·80	461	Survived
D	June 24—July 9	16	17·09	1068	Died with bracken poisoning.
	TOTAL ...	72	42·89	596	
G	Mar. 16—May 24	70	42·86	612	Died with bracken poisoning.

was fed during the intervening periods, as will be described later, but as this produced no symptoms the feeding of chopped dried bracken was recommenced.

FIG. 3



LIVE WEIGHTS AND FOOD CONSUMPTION IN DRIED AND CUBED BRACKEN FEEDING EXPERIMENTS

Sheep C, which had not eaten fresh bracken readily and had since been fed largely on hay, was starved for several days before commencing to feed on the dried bracken. Food consumption was low at the beginning of the experiment and body weight fell, but later increased gradually. Chopped dried bracken was fed to

this animal in three separate periods of 38, 5 and 33 days respectively. Between the first two of these periods there was an interval of 44 days when the principal feed was cubed, alcohol-extracted bracken, consumed at the rate of about 2 Kg. daily, with the addition of a small amount of chopped dried bracken to provide material for rumination; only 50 to 200 g. of the chopped dried bracken were consumed daily. Between the last two periods there was an interval of 10 days when cubed whole bracken formed the sole diet and an average of 172·7 g. of this were consumed daily.

It will be seen from Table II that the consumption of dried bracken by sheep C was low during the first period but rose to a satisfactory level in the later periods. Throughout all three feeding periods on dried bracken and also in the two periods when cubed bracken was fed, no abnormality developed in sheep C, except for low temperatures (Figure II) and the presence of a little mucus in the faeces. When bracken feeding was terminated on August 10th this animal appeared quite healthy and up to August 7th it was still gaining weight. From August 11th sheep C was given hay *ad lib* and on the morning of August 17th, after 6 days' feeding, showed the typical symptoms of bracken poisoning. No record of temperature was obtained on that date and the animal soon succumbed. Post-mortem examination showed the typical areas of congestion and petechial haemorrhages on the walls of the abomasum and also in the small intestine and caecum. Haemorrhages were also seen in the liver, lungs, heart, and spleen and on the walls of the bladder. No toxins of enterotoxaemia were found in the bowel contents.

Sheep D was fed chopped dried bracken in two separate periods of 56 days and 16 days respectively. Between these two periods there was an interval of 44 days during which cubed whole bracken was consumed at an average rate of 1381 g. per day. Apart from low temperatures and the presence of a little mucus in the faeces no abnormality was seen until the end of the experiment. The temperature ranged between 102·0° F. and 103·5° F. during most of the time but rose to 104·4° F. towards the end of the experiment. In the 72 days of bracken feeding sheep D ate an average of 596 g. per day. During the first 24 days, however, the bracken consumption was very small, averaging only 83 g. per day and the live weight consequently showed a marked fall (Figure III). Subsequently, however, the daily consumption averaged 852 g. which is a much more satisfactory level and sufficed to produce an almost continuous increase in live wieght. On July 9th, after a total of 72 days feeding on chopped dried bracken plus 44 days on cubed whole bracken, sheep D refused its food and the temperature rose suddenly to 109° F. (Figure II). The faeces were very soft with much mucus and some blood clots. Death occurred the same day with slight haemorrhages on the gums, congestion on one eyelid and much blood at the anus. At post-mortem exam-

ination the whole contents of the abomasum appeared to be blood and the walls were completely covered with petechial haemorrhages. Extensive diffuse and petechial haemorrhages were also found on the walls of the small intestine, caecum and colon, and there were some areas of congestion beneath the skin of this animal. Haemorrhages were present in the heart, lungs, liver and spleen. No toxins of enterotoxaemia were found in the bowel contents.

Sheep G was fed chopped dried bracken for an unbroken period of 70 days and the average consumption was very similar to that of sheep D. During the initial period of low food consumption there was a marked fall in body weight but this was followed by a continuous rise associated with an increasing food consumption (Figure III). Although the average dried bracken consumption for the whole experiment was only 612 g. per day, during the later part of the experiment, prior to the development of bracken poisoning, the intake was about 1 Kg. per day. Dried bracken was fed for two months (March 16th to May 15th) without any abnormality except a low temperature, but on May 16th some gelatinous material and blood clots were observed in the urine and mucus in the faeces. No further abnormality was noticed until May 20th when the temperature rose to 106·9° F. (Figure II) and remained high until May 24th, varying between 105·4° F. and 106·6° F. From May 21st to 24th the faeces were very soft, had a foetid odour and contained blood clots. On the latter date this sheep was found dead with the typical post-mortem picture of bracken poisoning; i.e. extensive congestion and small haemorrhages on the walls of the abomasum and intestines, some subcutaneous diffuse haemorrhages and extensive haemorrhages in heart and lungs. No toxins of enterotoxaemia were found in the bowel contents.

THE FEEDING OF CUBED BRACKEN

Since both fresh and artificially dried bracken were not palatable to sheep and difficulties were encountered in obtaining adequate consumption of the plant, some of the artificially dried bracken was hammer-milled and cubed in the hope that an increased consumption would be achieved and a more rapid toxic effect produced. For another experiment a quantity of the bracken meal was extracted with alcohol in a commercial plant and the residual extracted meal was also made into cubes for feeding purposes. The cubed bracken was fed to sheep C and D after the initial feeding with chopped dried bracken for 38 and 56 days respectively (see Table II) and had not produced any effect. As the cubed bracken also failed to produce any symptoms the feeding of chopped dried bracken was later resumed. The quantities of cubed bracken consumed in these experiments are given in Table III and the live weight data are included in Figure III.

Sheep C was fed the cubed alcohol-extracted bracken for 44 days without any untoward effect. Indeed the alcohol extraction rendered the bracken much more palatable and these cubes were consumed in considerable quantity. After seven days the sheep was eating 2 Kg. per day and making considerable live weight gains (Figure III). A small amount of chopped dried bracken was

TABLE III

CUBED BRACKEN FEEDING EXPERIMENTS.

Sheep	*Duration of Experiment*	*No. of days*	*Type of cubes fed*	*Cubed bracken consumed*		*Chopped dried bracken consumed*		*Result*
				Total	*Average per day*	*Total*	*Average per day*	
C	May 11—June 23	44	A	85·93 Kg.	1953 g.	4·83 Kg.	110 g.	Survived.
C	June 29—July 8	10	B	17·27 Kg.	1727 g.	Nil	Nil	Survived.
	Total ...	54	—	103·20 Kg.	1911 g.	4·83 Kg.	89 g.	—
D	May 11—June 23	44	B	60·78 Kg.	1381 g.	9·06 Kg.	206 g.	Survived

A = alcohol extracted bracken.
B = whole bracken.

also offered each day to provide material for rumination, but this was very largely refused. From Table III it is seen that about 86 Kg. of extracted bracken cubes were eaten in 44 days, this being nearly double the quantity of dried bracken which, when eaten during a considerably longer period, caused the death of sheep G. Nevertheless sheep C showed no ill-effect of any sort. For the short period of 10 days sheep C consumed whole bracken cubes

without the addition of any chopped dried bracken at all. Although the whole bracken cubes were not so palatable as the extracted bracken cubes the average level of consumption shown in Table III was still very high and no harmful effect on the animal was apparent. That this animal was capable of developing bracken poisoning was shown by the further 33 days of feeding on chopped dried bracken which followed the feeding of the cubes (Table II) and produced the typical case of bracken poisoning described above.

Sheep D was given whole bracken cubes along with 250 g. chopped dried bracken daily for 44 days. Although the consumption of these cubes was below the level at which sheep C ate extracted bracken cubes, the average daily intake of 1381 g. was very satisfactory and more than could be achieved with chopped dried bracken alone. Although 43 Kg. dried bracken consumed in 70 days (average 612 g. per day) caused the death of sheep G and the same amount consumed in 72 days (average 596 g. per day) also caused the death of sheep D (Table II), 61 Kg. of whole bracken cubes consumed in 44 days (average 1381 g. per day) had no harmful effect.

DISCUSSION

The experimental feeding of fresh bracken to sheep has shown that there may be considerable difficulty in securing an adequate consumption and that in this respect there may be marked differences in the behaviour of different animals, some sheep being much more ready to eat bracken than others. Nevertheless, when it is possible to induce the consumption of fresh bracken in sufficient quantities, as in the case of sheep F, experimental bracken poisoning can be produced, with the same symptoms and post-mortem findings as in cattle. The production of the disease in sheep F appears to be the first clear case of typical bracken poisoning in sheep recorded in this country and it is concluded that the only reasons the condition does not commonly occur in sheep, as in cattle, are their more selective feeding habits and their reluctance or inability to consume bracken in sufficient quantity. Sheep F, which developed bracken poisoning, consumed three times as much bracken as sheep E, which, although it succumbed, did not die from typical bracken poisoning. For the experimental production of poisoning with fresh bracken the use of sheep merits consideration, these animals being cheaper and easier to handle and feed than cattle. It is advisable, however, to have a number of sheep available so that those which prove unsatisfactory as regards the consumption of bracken may be discarded.

The production of bracken poisoning in three sheep fed on artificially dried bracken indicates that the preservation of bracken by artificial drying may offer a means of extending considerably the experimental work on this problem. It will be noted, however, that large quantities of dried bracken had to be fed before

any symptoms appeared. Although sheep F, in which bracken poisoning was produced by feeding fresh bracken, consumed 33 Kg. of bracken dry matter in a period of 66 days, considerably greater quantities of dried bracken were needed to produce poisoning in sheep C. D and G, which were fed for only a slightly longer time. It appears that larger quantities of bracken dry matter may need to be consumed to produce toxicity when dried bracken is fed than when fresh bracken is used and if this is so, the conditions of drying will obviously be of importance. The temperature of the drying gases may be of little significance as the bracken itself will be cooled by the evaporation of moisture during the process; exposure of the bracken to high temperatures after it is dried, however, may have quite a marked effect on its toxicity. Besides enabling experimental feeding to be carried out at any convenient time the use of artificially dried bracken in place of fresh bracken was found to lead to a considerably increased daily consumption of bracken dry matter. However, the apparent effect of heating in lowering the level of toxicity and increasing the quantity necessary to produce bracken poisoning needs careful consideration since if overheated material should be of greatly reduced or negligible toxicity a considerable amount of time and effort would be wasted in feeding it.

Cubes prepared from alcohol-extracted bracken were found to be quite palatable to sheep and to have no poisonous effect even when fed at a high level for 44 days. The live weight gains of sheep C, shown in Figure III, indicate that these cubes had a very definite nutritive value. Cubes prepared from whole bracken were less palatable but nevertheless eaten in considerable quantity by sheep D without apparent effect. This unexpected result is not readily explained although it may be that the second heating involved in the cubing process had some effect on the toxicity. It is also possible, as has been suggested by previous workers, that the irritation of the gastro-intestinal tract produced by long bracken may be necessary for the absorption of the toxic factor. Although cubing offers a means of securing a very considerably increased consumption of bracken, the failure to produce bracken poisoning by the feeding of very large amounts rules out this as a practical experimental procedure.

SUMMARY

Bracken poisoning has been produced experimentally in sheep by feeding fresh bracken, although it was found difficult to induce sheep to eat adequate quantities of the plant. The apparent absence of natural cases in sheep is probably due to their selective grazing habits. Bracken poisoning in sheep produced the same symptoms and post-mortem findings as in cattle.

The poisoning of ruminants fed on bracken appeared to be closely related to the stage of growth, young bracken being most toxic.

Bracken poisoning was also produced in sheep by feeding artificially dried bracken, although a greater consumption of dry matter was found to be necessary when this material was used. There were indications that the drying process may reduce the degree of toxicity of the plant.

Whole bracken cubes did not show any poisonous effect when fed to sheep at a relatively high level (1·38 Kg. per day) for 44 days. Cubed alcohol-extracted bracken fed to a sheep for a similar time and in even larger amounts (1·95 Kg. per day) had no poisonous effect and was quite palatable.

The live weights of sheep fed entirely on bracken were closely related to the quantities of dry matter consumed. When sufficient bracken (fresh, dried or cubed) was consumed its nutritive value was clearly demonstrated by the gains made.

ACKNOWLEDGMENTS

The authors are indebted to Messrs. J. T. Stamp, J. A. Watt, and D. I. Nisbet, Veterinary Investigation Officers, for assistance with some of the post mortem examinations and for carrying out the tests for enterotoxins. Thanks are also due to Professor S. J. Watson and the University of Edinburgh for the provision of a grant which enabled this work to be carried out, and to Messrs. John Wilson and Co., Ltd., Leith, for preparing the bracken cubes.

REFERENCES

Evans, W. C., and Evans, E. T. R. (1949). *Brit. vet. J.*, **105,** 175.
Evans, W. C., Jones, N. R., and Evans, R. A. (1950). *Biochem. J.*, **46,** xxxviii.
Fletcher, J. M. (1944). *Vet. Rec.*, **56,** 478.
Haag, J. R., Weswig, P. H., and Freed, A. M. (1947). *Fed. Proc.* **6,** 408.
Haag, J. R., Weswig, P. H. (1948). *Ibid.*, **7,** 157.
Hickman, A. J. (1910). *N.Z. J. Agric.*, **1,** 215.
Moon, F. E., and Pal, A. K. (1949). *J. agric. Sci.*, **39,** 296.
Roberts, E., Evans, E. T. R., and Evans, W. C. (1949). *Vet. Rec.*, **61,** 549.
Shearer, G. D. (1945). *J. comp. Path.*, **55,** 301.
Stamp, J. T. (1947). *J. British Grassland Soc.*, **2,** 191.
Stockman, S. (1917). *J. comp. Path.*, **30,** 311; (1922). *Ibid.*, **35,** 273.
Thomas, B., and Walker, H. F. (1949). *J. Soc. chem. Ind.*, **68,** 6.
Weswig, P. H., Freed, A. M., and Haag, J. R. (1946). *J. biol. Chem.*, **165,** 737.

[*Received for publication, November 15th,* 1950.]

STUDIES ON RESPIRATORY DISEASES OF PIGS

III.—Persistence of Influenza Viruses in the Respiratory Tract

By

T. S. Gulrajani*

Department of Animal Pathology University of Cambridge.

INTRODUCTION

It is generally considered that in human influenza the virus normally cannot be detected in the respiratory tract for more than about a week after the onset of the disease and that carriers beyond this period, if they occur at all, are rare. On the other hand, there has been a rather widespread belief in this country and on the Continent of Europe that in swine influenza the virus commonly persists in pigs for many weeks and probably months after initial infection (Blakemore and Gledhill, 1941a; Hjarre, Bakos and Nordberg, 1949). Since the virus of swine influenza is closely related to the human virus and since natural infection of swine with human strains has been reported (Shope, 1938; Shaw, Kenney and Stokes, 1939; Dreguss, 1945) it is important from the point of view of public health as well as of animal health to have definite information regarding the persistence of the virus of influenza (either swine or human strains) in the respiratory tract of pigs. The matter was therefore investigated, but no evidence was obtained that the virus persists in pigs longer than it does in man.

MATERIALS AND METHODS

Strains of influenza virus.—In all experiments with swine influenza the strain C.1. obtained at Cambridge by Blakemore and Gledhill (1941b) was used. This strain was isolated in ferrets and, after ten passages in this animal, was adapted to mice by Glover and Andrewes (1941). Thereafter its exact history is not known, but it had at least one chick embryo passage before it was received at this laboratory in the form of dried mouse lungs and had retained its full virulence for pigs. Here it has been maintained by passage in mice. The usual material for inoculation of pigs and mice consisted of a 1 in 20 suspension of the lungs of mice which had been inoculated two days before they were killed.

When this strain was re-isolated in chick embryos from pigs' lungs in this laboratory, the ratio of haemagglutination titres of the amniotic fluids with fowl and guinea pig r.b.c. was usually 0·3 to 0·5 whereas after several passages this ratio became 1 or greater, which suggests that the strain had not previously been fully adapted to chick embryos. (Burnet, Beveridge, Bull and Clark, 1942).

* Under a grant from the Agricultural Research Council.

A recently isolated strain of human influenza A from an epidemic in Holland in 1949 was kindly supplied by Dr. C. H. Andrewes in the form of infective allantoic fluids. Four ampoules of early passage virus, two each from persons "H" and "B" both from the same outbreak, were received. The titres of these fluids were twice as high when titrated with guinea pig r.b.c. as with fowl r.b.c., showing that the virus had undergone little modification from its original form.

Pigs.—Most of the pigs were bred on this Department's farm. Some, however, were purchased from farms believed to be free from swine influenza.

For inoculation, pigs were anaesthetised with ether and held in a vertical position. With a graduated pipette, 10 ml. of inoculum was slowly instilled into the nostrils. Temperatures were recorded twice or thrice daily.

The method of taking nasal washings was to inject 10 ml. of sterile broth into one of the nostrils with a syringe and rubber tube. As the broth ran out it was collected and re-injected into the other nostril and collected again. As a regular routine, samples of sera were taken from pigs before inoculation and twelve days after and tested for the presence of antibody against the homologous strain of virus by the modified haemagglutination-inhibition (H.I.) test described earlier (Gulrajani, 1951a).

Mice.—For intranasal inoculation, mice were anaesthetised with a mixture of chloroform 1 part, ether 2 parts, according to the technique described by Burnet and Timmins (1937). To test material for the presence of virus, a group of six white mice, 6 to 8 weeks old, was inoculated intranasally each with 0·05 ml. Two of these mice were killed after two days, and those surviving were killed on the 7th day. As a routine, a 1 in 10 broth suspension of lungs of mice killed at two days was inoculated into four more mice and into the allantoic cavity of chick embryos. The mice in the second group were also killed on the 7th day. If consolidation of lungs was not seen in the two batches of mice thus inoculated and the allantoic fluid of the chick embryos did not agglutinate fowl or guina pig r.b.c., the original inoculum was considered non-infective.

Lesions in the lungs of mice were recorded as follows: + + + + = complete consolidation, + + + = three-fourths of lungs consolidated, + + = half consolidated, + = one-quarter consolidated, − = no consolidation.

Tracheal washings of mice were taken after killing by inserting a blunted hypodermic needle into the trachea and tying it there with a silk thread: 0·2 ml. of sterile distilled water was injected and sucked back three times.

Ferrets.—Ferrets were bought from local breeders and quarantined for at least 14 days. They were inoculated intranasally after anaesthetising with ether. The dose was 1 to 2 ml. according to the

size of the animal. When there was any suspicion of the presence of bacteria in the inoculum, antibiotics were added. Temperatures were recorded twice daily. From each ferret nasal washings were taken two days after inoculation, using the technique described by Burnet and Büll (1943). These washings were inoculated into the amniotic cavity of chick embryos as a regular routine. Also as a routine, serum samples were taken from each ferret before inoculation and 12 days after, and titrated by the modified H.I. technique for the presence of antibody.

Chick embryos.—To test material for the presence of virus, 13 to 14 day embryos were inoculated by the amniotic route in batches of four to eight by the technique of Beveridge (1947). Those dying within two days were discarded, and some of the remainder were opened on the 3rd and some on the 4th day. The amniotic fluid in each case was tested with fowl and guinea pig r.b.c. and a suspension of embryonic lungs was similarly tested. If there was no haemagglutination, a pool of amniotic fluid from at least two eggs was inoculated into a further batch of embryos. If amniotic fluids and embryonic lung tissue suspension from the 2nd batch of embryos also gave no haemagglutination, the original inoculum was considered not to contain influenza virus.

When pig or mice lungs were used as a source of inoculum for chick embryos, small pieces of lung from six different places were ground and suspended in broth. The suspension was lightly centrifuged and the supernatant used as inoculum. As a routine, penicillin and streptomycin were added to give a concentration of 3,000 units of each per ml. and each embryo was injected with 0·1 ml.

Bacteriology.—As a routine, cultures from four different sites on the cut surface of each lung and from at least 4 bronchioles were made on chocolate and blood agar plates. Agar slopes, to the bottom of which 0·5 ml. of sterile horse blood was added, were used sometimes instead of chocolate plates. Most of the lungs, however, were bacteriologically sterile and *Haemophilus influenza suis* was not recovered from any of them.

SWINE INFLUENZA VIRUS IN PIGS

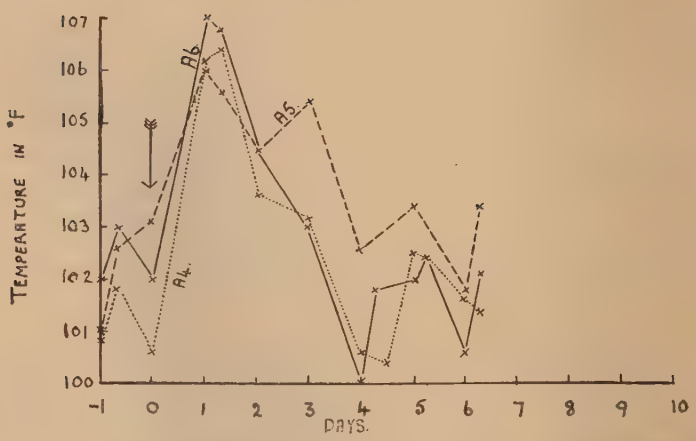
Attempts to recover virus from 3rd to 68th day after inoculation

Pig inoculations.

A litter of seven pigs six weeks old, numbered A1 to A7, was inoculated intranasally with a 5 per cent. mouse lung suspension of strain C.1 Temperatures of three of these pigs are shown in Graph 1.

On the day after inoculation a rise in temperature of at least 3°F. was recorded in all pigs. They appeared somewhat depressed and had slight difficulty in breathing but fed normally. Two days after inoculation they were very listless, fed very little, appeared

GRAPH I



GRAPH No. 1. Temperature curves of pigs (A4, A5 and A6) inoculated on day 0 with virus of swine influenza.

extremely uneasy and had rough coats. The temperatures had decreased, but were still well above normal. They spent most of the time in a corner and had the appearance of feeling very cold, although their pens were fairly warm. Pig A_5 coughed very often.

On the morning of the 3rd day pig A_1 was found dead. From the 4th day onwards the temperature and breathing of the remaining pigs became normal and within a week's time all the pigs were quite normal. H.I. titres of sera from these pigs have been reported elsewhere (Gulrajani, 1951b).

Pig A1. On autopsy this pig, which died on the 3rd day after inoculation, was found to have extensive areas of pneumonia in all lobes, the other organs appearing normal. Virus was recovered by inoculation of mice with a suspension of the pig lungs. The haemagglutination titre of the mouse lung suspension was 160 with fowl r.b.c. and 320 with guinea pig r.b.c.

Pigs A2 to A7. One of the remaining six pigs was killed on the 28th day, one on the 53rd, one on the 61st and two on the 68th day after inoculation. One died of bowel oedema on the 53rd day. Pneumonia was present in each pig.

A 1 in 10 broth suspension of each lung was inoculated into a ferret, mice and chick embryos. In addition, suspensions of lungs of the four pigs killed on the 53rd day or later were each inoculated into a pig. In no instance was the virus detected. None of the pigs showed antibody by the H.I. test. Three of the four pigs (C_9, D_1, D_2) used for testing the presence of virus in these experiments, were subsequently shown to be susceptible to swine influenza infection, as is described in the following experiment.

Experiments to determine sensitivity of different methods of recovering virus

The failure to demonstrate virus 28 to 68 days after inoculation was not in accordance with expectations based on previous work in this country. The following experiment was therefore conducted to check the methods used for detecting the virus and to compare the relative sensitivity of these methods.

Pig inoculations

Pig C9 was inoculated with a 5 per cent. suspension of infected mouse lung. This pig showed a rise of temperature and other symptoms closely similar to those described under the previous experiment. It was killed on the 4th day and a 1 in 10 suspension of its lungs was inoculated into pig D1, ferret 14, mice and chick embryos. After the lungs of pig C9 had been stored for five days in 50 per cent. glycerol at 0° C., pig D2 was also inoculated.

Ferrets

Ferret 14 showed no appreciable rise of temperature, but on the 7th day it was coughing and sneezing and refused food. Virus was re-isolated from the nasal washings taken two days after inoculation. Antibody titres of the ferret are shown in Table 1.

Mice

Of the mice inoculated with pig lung suspension only one showed + lesions when killed on the 7th day. In the second passage group, two had + + + + lesions and two + + lesions. Virus was re-isolated in chick embryos from the first passage mice which were killed on the 2nd day. The allantoic fluids had fowl/guinea pig titres of 20/60 and 15/20.

Chick embryos

Virus was detected in the chick embryos inoculated with a 1 in 10 suspension of lungs of pig C9. Subsequently 10^{-2} and 10^{-3} suspensions of the lungs were inoculated into chick embryos. The virus was recovered from each of these suspensions. The fowl/guinea pig ratio of two out of three fluids tested was 0·5, while the ratio of the 3rd fluid was more than one.

Pigs

Pig D1 showed no rise of temperature or any other symptoms of infection within five days of inoculation. In case the failure of pig D1 to react was due to its being immune as the result of a previous inoculation (see previous experiment), on the 5th day a suspension of the lungs of pig C9 was inoculated into a further pig, D2. *Pig D2* also showed no appreciable rise of temperature or any other symptoms of infection. Antibody was, however, detected in the post-inoculation samples of sera of both pigs (see Table 1). The most likely explanation for these infections being sub-clinical is that the inoculum contained insufficient virus to produce clinical infection.

TABLE I

RECOVERY OF VIRUS FROM LUNGS OF PIG C9, KILLED 4 DAYS AFTER INOCULATION.

Inoculation into pigs				*Inoculation into ferret*				*Inoculation into mice*		*Inoculation into chick embryos*		
Pig No.	*Symptoms*	*H.I. titres*		*Symptoms*	*Nasal washings into chick embryos*	*H.I. titres*		*1st pass*	*2nd pass*	*Dilutions*		
		pre	*post*			*pre*	*post*			10^{-1}	10^{-2}	10^{-3}
D1	None	<10	160	Slight	positive	<10	15360	slight lesions in 1 mouse	Lesions in all mice	positive	positive	positive
D2	None	20	100									

Pigs, ferret and mice were inoculated with a 1 in 10 suspension.

Pre = pre-inoculation serum. post = post-inoculation serum. pass = passage. positive = virus detected.

Advantage was taken of this opportunity to see whether the virus persisted in a pig which had had a sub-clinical infection. Pig D2 was killed 16 days after inoculation and a suspension of its lungs inoculated into a ferret and into chick embryos. No virus was detected.

Comment

The results obtained by the different methods of detecting swine influenza virus in this experiment and summarised in Table 1 show that a sub-clinical infection was set up in pigs D1 and D2 inoculated with a 1 in 10 suspension of lungs from pig C9. Ferret 14 also showed no rise of temperature but developed some clinical symptoms on the 7th day. Only slight consolidation of the lungs of one mouse was present in the first batch of mice, although consolidation was seen in lungs of all the mice in a further passage. The results suggest that the virus would not have been detected in higher dilutions by inoculation of these animals. Yet the amniotic cavity of embryonated eggs detected the virus in a 10^{-3} suspension of pig lungs, i.e. a 1/100th dilution of the inoculum used for pigs, ferret 14 and mice. This strongly suggests that injection by the amniotic route of 13 to 14-day chick embryos is the most sensitive method of detecting swine influenza virus strain C.1, This technique also has the advantage that it is at least as simple as the other techniques and gives quicker results. Amniotic inoculation of chick embryos is now used as a standard technique for isolation of human influenza virus and has replaced the use of ferrets in most laboratories.

Attempts to recover virus at short intervals after inoculation

The results of the above experiment showed that the methods used for detecting the virus were satisfactory. In the experiments so far described the virus was recovered from one pig dead on the 3rd day and from one killed on the 4th day, but not from a sub-clinical case killed on the 16th day or from clinical cases killed on the 28th day or later after inoculation. It was, therefore, decided to kill pigs at short intervals from the 4th day of inoculation onwards.

Pig inoculations

Pig F1 was inoculated with a 5 per cent. broth suspension of mouse lungs infected with strain C.1.; 24 hours later the temperature rose to 107° F. It showed the usual symptoms of swine influenza and was killed on the 7th day after inoculation. A suspension of 1 in 10 of its lungs was inoculated into ferret 16 and into the amniotic cavity of chick embryos. No virus was detected.

A batch of four pigs eight weeks old (51, 52, 53, 54) was inoculated with a 5 per cent. suspension of infected mouse lungs. The pigs showed the usual rise of temperature and other symptoms typical of swine influenza.

Pig 54 was killed on the 4th day after inoculation. A suspension of 1 in 10 of lungs of this pig was inoculated into ferret 17, into

mice and into chick embryos. The virus was recovered by each method.

Various dilutions of a suspension made from portions of lungs of pig 54 after they had been stored for a week in 50 per cent. glycerol buffer were titrated in the amniotic cavity of chick embryos. The virus was recovered in all dilutions tested, that is 10^{-1} to 10^{-5} (see Table II).

TABLE II

RESULTS OF INOCULATION INTO CHICK EMBRYOS OF SUSPENSION OF LUNGS OF PIG 54.

Suspension of lungs	*Results*	
	3rd day	*4th day*
10^{-1}	+,+,+	+,+,+,+,−
10^{-2}	+,+	+
10^{-3}	+,+,+	+
10^{-4}	+	+,+,−
10^{-5}	−	+,+

Each + or − represents a chick embryo.
+ = Virus recovered.
− = Virus not recovered.

Pig 51. Nasal washings were taken from pig 51 on the 2nd, 6th and 9th days and the pig was killed on the 10th day after inoculation. Virus was readily detected by chick embryo inoculations with washings taken on the 2nd and 6th days, but not on the 9th day.

Pig 52. Nasal washings were taken from pig 52 on the 2nd and 6th days and the pig was killed on the 10th day after inoculation. Virus was re-isolated in chick embryos from the washings taken on the 2nd day but not from those taken on the 6th day. The washings taken on the 2nd day also yielded virus when a 1 in 10 dilution was inoculated into chick embryos.

Pig 53. Nasal washings were not obtained from pig 53. The pig was killed 10 days after inoculation, along with pigs 51 and 52.

Pigs 51, 52, 53. Separate suspensions of 1 in 10 made from the lungs of each pig were inoculated into mice and into chick embryos, but the virus was not detected. Ferrets 18 and 19 were also inoculated with suspensions from the lungs of pigs 51 and 53 respectively, but these also failed to reveal any evidence of influenza virus.

In this experiment, the virus was recovered only from the lungs of a pig killed on the 4th day, but not from the lungs of three pigs killed on the 10th day. The virus was detected in the nasal washings of pigs taken on the 2nd day and from one of the two pigs on the 6th day, but not from washings taken on the 9th day.

The results obtained in the earlier experiment suggesting that inoculation into the developing egg was more sensitive than the other methods used for detecting the virus, received further support from the results of this experiment where virus was detected in pigs' lungs even at a dilution of 10^{-5} by chick embryo inoculations. In view of these findings only inoculation of the amniotic cavity of chick embryos was used for detecting virus in all further experiments. It was also found that when amniotic fluids caused haemagglutination, lung suspensions of only some of these embryos gave haemagglutination. On the other hand, in no case did embryonic lung suspensions give haemagglutination when the amniotic fluids did not and therefore in further experiments only amniotic fluids were tested for agglutination of red blood cells.

Pig inoculations

Another experiment was carried out on the same general lines as the previous experiment. Two ten-week-old pigs (55 and 56) were each inoculated with a 5 per cent. suspension of infected mouse lungs. The pigs showed the rise of temperature and other symptoms typical of swine influenza.

Pig 55. Nasal washings were taken from this pig on the 3rd, 5th, 7th and 9th days, and the pig was killed on the 11th day after inoculation. The virus was detected from washings taken on the 3rd and 5th days only, and it was not detected in those of the 7th day; those of the 9th day were not tested. The washings taken on the 5th day were infective for chick embryos when diluted 10 times. The virus was not recovered from the lungs of the pig when killed on the 11th day.

Pig 56. The virus was detected by chick embryo inoculation from nasal washings of this pig taken on the 4th day in dilutions of up to 10^{-2}. This was the highest dilution tested. Unfortunately, the pig died on the 5th day as a result of the swine influenza inoculation. The virus was recovered from its lungs in suspensions of up to 10^{-3} which was the highest dilution tested.

Attempts to recover virus after inoculations of pigs with a small dose alone or together with bacteria

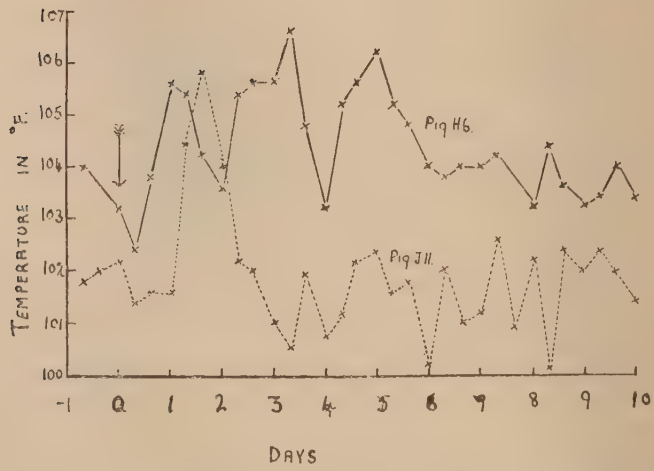
The experiment just described gave results similar to those obtained in earlier experiments. The acute disease set up in pigs used in the above experiments had stimulated a high titre of antibody which may have militated against the persistence of the virus or against its detection. It will, however, be recalled that the virus was not recovered on the 16th day from pig D2 after a sub-clinical infection, as reported earlier. In this experiment a pig was inoculated with a one tenth concentration of the usual inoculum, to see whether a less severe infection could be set up which might allow the virus to persist longer in the pig's lung.

Pig inoculation

Pig J11 (twelve weeks old) was inoculated with a 0·5 per cent.

suspension of infected mouse lungs. The pig showed a definite rise of temperature (Graph 2), but no other clinical symptoms. Nasal washings of this pig were taken on the 2nd and 10th days and inoculated into chick embryos. The virus was recovered only from those taken on the 2nd day. The pig was killed on the 10th day after inoculation. A very small area of pneumonia was present in the left apical lobe. A 1 in 10 suspension of lungs was inoculated into chick embryos, but the virus was not detected.

GRAPH 2.



GRAPH No. 2. Temperature curves of pigs inoculated on day 0 with swine influenza virus alone (J11) or virus plus bacteria (H6).

The reports of isolation of virus from field outbreaks a long time after infection of pigs have mostly been from chronic cases. In the experiments so far described, the disease set up in the pigs was of short duration and nearly all the lungs were bacteriologically sterile. It seemed possible that the addition of secondary bacteria in the inoculum might result in a prolonged chronic infection in which the virus might persist longer than in acute cases which recover rapidly. In this experiment, therefore, pigs were inoculated with a mixture of virus and bacteria.

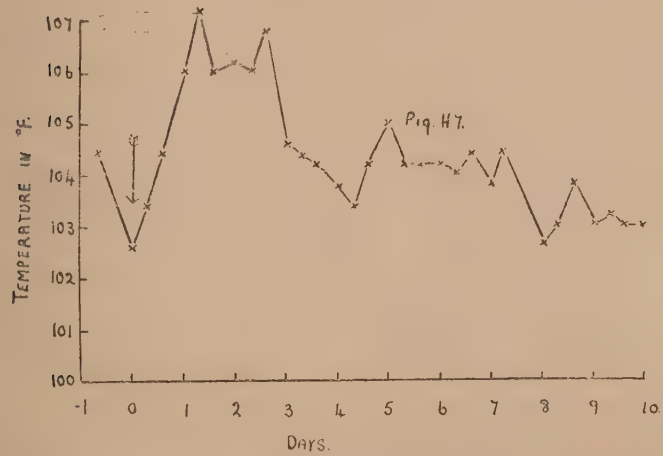
Pig inoculations

Pig H6 (twelve weeks old) was inoculated intranasally with 10 ml. of a 0·5 per cent. suspension of infected mouse lungs to which was added 1 ml. of *Bact. friedlanderi* suspension. The bacteria were grown on a blood agar plate for 24 hours and the growth on the

plate was scraped off and suspended in 5 ml. of plain broth. The strain of *Bact. friedlanderi* had recently been isolated from a pig which died in a field outbreak of pneumonia. *Pig H7* (twelve weeks old) was inoculated intranasally with 5 ml. of a 24 hours broth culture of a Pasteurella mixed with 5 ml. of a 1 per cent. virus suspension. The culture had recently been isolated from a field case of pneumonia and was subcultured only twice before it was used to inoculate the pig.

The temperature reaction in these two pigs (Graphs 2 and 3) was more severe than in pig J11 (Graph 2) although the amount of virus used for these three pigs was approximately the same, namely, 10 ml. of a 0·5 per cent. suspension of infected mouse lungs. The temperature reaction was at least as great as in the pigs which had been inoculated with a 5 per cent. suspension of virus alone (e.g. Graph 1).

GRAPH 3



GRAPH No. 3. Temperature curve of pig H7 inoculated on day 0 with swine influenza virus together with bacteria.

Pig H7 was slightly listless on the 3rd day. Otherwise the animals showed no clinical symptoms. Nasal washings were taken on the 10th day from both pigs which were killed on the 13th day after inoculation. Virus was not detected in the nasal washings of either pig. The lungs of pig H6 were bacteriologically sterile but a Pasteurella was grown from the lungs of pig H7. Virus was not detected in the lungs.

Summary of experiments on recovery of swine influenza virus in pigs.

The virus has been re-isolated from the lungs of four pigs and from six nasal washings taken within five days of inoculation and

from the nasal washings of one of two pigs on the 6th day. It was not recovered from five nasal washings taken on the 7th to 10th day, the lungs of one pig killed on the 7th day and the lungs of eight pigs killed on the 10th to 16th days after inoculation. The lungs of six pigs killed at 28 to 68 days also gave negative results.

TABLE III

DETAILS OF MICE INOCULATED WITH VARIOUS DILUTIONS OF SWINE INFLUENZA VIRUS.

Suspension used to inoculate mice	*No. of mice inoculated*	*Mice dead within 8 days*	*Mice killed and lesions*		
			8th day	*12th day*	*17th day*
10^{-3}	24	18	K+++++ K+++++	K+++ }* K++ }	K+++ }* K++ }
10^{-3}	12	5	K+++ K+++	K+ K— K—	K++ K+
10^{-4}	4	1	K++ K— K—		

Mice dead within 8 days were discarded. K = killed. ++++, +++, ++, + = degrees of consolidation of lungs.
— = Lungs normal. * Tracheal washings were obtained from these mice.

SWINE INFLUENZA VIRUS IN MICE

In the hope of obtaining information which might have a bearing on the results obtained in pigs, an experiment with mice was planned to obtain data on the following questions: (1) How long does the virus persist in mice?; (2) does it persist longer after

inoculation with a small dose than after a large dose?; (3) can it be detected after longer periods by washing out the trachea and bronchii than by grinding up the lungs?

Three groups of mice were inoculated with 10^{-2}, 10^{-3} and 10^{-4} suspensions of infective mouse lung respectively. Two days later, all these mice were inoculated with plain broth. Mice inoculated with 10^{-4} suspension were killed on the 8th day after inoculation. Two to three mice were killed from each of the groups inoculated with 10^{-2} and 10^{-3} suspensions on the 8th, on the 12th and on the 17th days.

In each group the lungs of mice killed on the same day were pooled and a suspension of 1 in 10 from the pool was inoculated into chick embryos. In addition, the lungs of mice inoculated with 10^{-2} suspension of virus and killed on the 8th day were inoculated into embryos in a dilution of 10^{-3}. Tracheal washings were taken from mice killed on the 12th and 17th days after inoculation with a 10^{-2} suspension of virus. Washings from mice killed on the same day were pooled and inoculated into chick embryos.

Further details of this experiment are shown in Table 3 and the results in Table IV. Although the numbers involved are too small to justify definite conclusions, there was no indication that the virus persisted appreciably longer after dilute inocula had been used to infect, nor did tracheal washings detect virus when it could not be demonstrated in lung suspensions. These results are in general agreement with the results obtained with swine influenza virus in pigs, and with the experiments reported by other workers in mice. (Burnet and Lush, 1938.)

TABLE IV

RESULTS OF INOCULATIONS OF MOUSE LUNGS IN CHICK EMBRYOS.

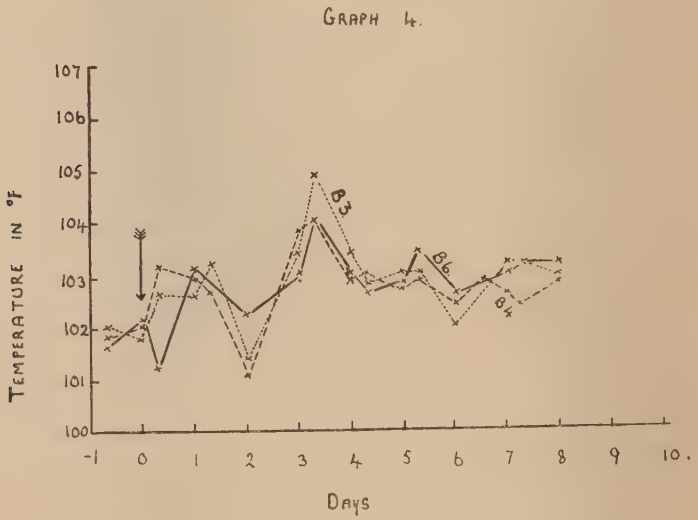
Suspension used to inoculate mice	*Time after inoculation that mice were killed*		
	8th day	*12th day*	*17th day*
10^{-2}	Positive*	Negative	Negative
		Tracheal washings (negative)	Tracheal washings (negative)
10^{-3}	Positive	Negative	Negative
10^{-4}	Positive		

Positive = Virus detected by chick embryo inoculations.
Negative = Virus not detected by chick embryo inoculations.
Separate pools were made of the lungs of mice which were killed on the same day from each group. From each pool of lungs a 1/10 suspension was inoculated into chick embryos.

* This pool of lungs was tested also at a final dilution of 1/1000 with positive results.

HUMAN INFLUENZA A VIRUS IN PIGS

Eight pigs, four weeks old (B1 to B8), were inoculated intranasally with a 1 in 20 dilution of early passage allantoic fluids as received from Dr. Andrewes (see Materials and Methods). Four of the pigs were inoculated with virus isolated from person "H", and others with virus from person "B". All the pigs showed a moderate rise of temperature on the 3rd day after inoculation, but normal temperatures were recorded the following day. The temperatures of three are shown in Graph 4. The pigs appeared

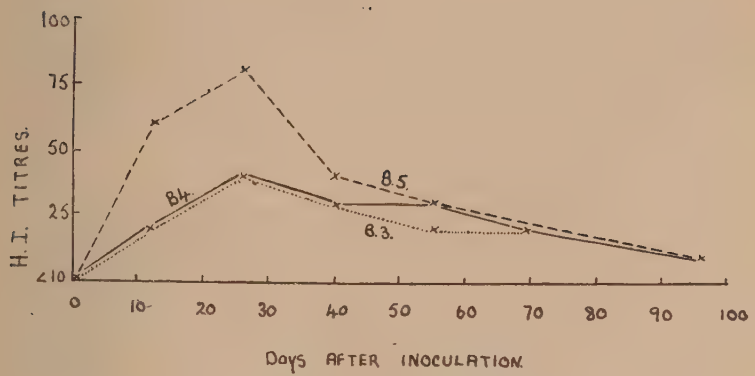


Graph No. 4. Temperature curves of pigs (B3, B4 and B6) inoculated on day 0 with virus of human influenza A.

slightly listless on the 3rd day, but they showed no other symptoms and ate normally. On the 7th day one pig was killed in order to test its lungs for virus. A sample of post-inoculation serum was taken from each of the remaining pigs as usual 12 days after inoculation. The sera of these seven showed antibody by H.I. titration against homologous virus. The titres of three over a period of two to three months are shown in Graph 5.

The pig which was killed on the 7th day had a small area of pneumonia in the margin of the left cardiac lobe. A suspension of the lungs was inoculated into the amniotic cavity of chick embryos and the virus was recovered. One more pig was killed each on the 28th, 39th, 46th and 52nd days after inoculation. The one killed on the 28th day showed a small marginal area of consolidation in the right cardiac lobe, but the lungs of the others appeared normal. Chick embryo inoculations failed to detect

GRAPH 5.



GRAPH No. 5. H.I. titres of sera from pigs (B3, B4 and B5) after experimental infection with virus of human influenza A.

virus in any of these lungs. The three remaining pigs were therefore discarded. The re-isolation of the human strain seven days after inoculation, but the failure to detect it 28 to 52 days later is in accord with the results obtained with swine influenza virus in pigs and mice.

DISCUSSION

Swine influenza virus has been recovered from the respiratory tract of pigs up to six days but not 10 or more days after inoculation, although pneumonia was present in all these pigs. Results of a similar nature were obtained with a recently isolated strain of human influenza A in pigs and also with swine influenza in mice. These results fit very well with the experience of investigators working with human influenza viruses in man, ferrets and mice, and with Shope's experience with American strains of swine influenza (Shope, 1941).

It is therefore concluded that the strains of influenza virus studied are not usually recoverable from the respiratory tract of pigs on the 10th day or later after inoculation, that is, about the time that antibodies are demonstrable in high titre in the circulation. It is likely that this conclusion applies equally to other strains of swine and human influenza in the pig, especially in view of the analogous experience of other investigators with man, ferrets and mice.

This conclusion, however, is in disagreement with the opinion of certain workers that in swine influenza the virus can commonly be isolated for long periods (Blakemore and Gledhill, 1941a; Hjarre

et al., 1949). The explanation of this conflict of views is probably two-fold: —

(1) Swine influenza has been confused with another infection of pigs' lungs, "infectious pneumonia of pigs", which in reality is a distinct disease and probably also due to a virus (Gulrajani and Beveridge, 1951). Swine influenza has perhaps also been confused with ferkelgrippe, the relationship of which to infectious pneumonia of pigs has not yet been determined. In these two latter diseases, the lesions in the lungs macroscopically resemble swine influenza and the infectious agent persists longer than in swine influenza.

(2) The results reported in this paper do not conflict with the idea that in swine influenza the virus might occasionally persist for periods of longer than 10 days. It is well known that in some virus diseases a small percentage of recovered cases remain carriers long after the great majority of animals have ceased to harbour the virus and this might be so with swine influenza. Shope's observations that swine influenza virus may persist for long periods in a "masked" form in lung worms are not relevant to this study as lung worms were not present.

The strain of swine influenza used in these experiments was a laboratory strain adapted to ferrets and mice. There is the possibility that it did not behave exactly as would a field strain but it was, however, still fully virulent for pigs. Blakemore and Gledhill (1941) reported the isolation of a strain of swine influenza from a pig eight weeks after the onset of the disease. It is noteworthy that this animal was exceptional in that it had a protracted illness, which is not usual with swine influenza, and was also infected with lung worms. Either or both of these factors would quite possibly have a bearing on the prolonged presence of the virus.

SUMMARY

Chick embryos, 13 to 14 days old, have been shown to be very susceptible to infection with the virus of swine influenza when inoculated into the amniotic cavity. Such inoculation was found to be a more sensitive method of detecting the C.1. strain in pigs' lungs and nasal washings than inoculation of pigs, ferrets or mice.

Experiments to ascertain the persistence of swine influenza virus in pigs have shown that a mouse adapted strain (C.1.) could be detected in nasal washings and lung suspensions of pigs only up to a week after inoculation. These results were obtained both when a clinical or a subclinical disease was produced by the virus alone, or when bacteria were mixed with a small inoculum of virus. A small experiment in mice gave similar results.

A mild disease was set up in pigs by the inoculation of a recently isolated strain of human influenza A. The virus was recovered from a pig killed on the 7th day, but not from pigs killed on the 28th day and thereafter.

ACKNOWLEDGMENTS

I am grateful to Dr. C. H. Andrewes who kindly supplied the influenza virus strains used in these experiments, and also to Mr. A. O. Betts for his help in the preparation of Graphs.

It is a great pleasure to acknowledge my indebtedness to Professor W. I. B. Beveridge for his supervision and help in the experiments described and his assistance in the final preparation of this series of papers.

REFERENCES

Beveridge, W. I. B. (1947). *Science,* **106,** 324.
Blakemore, F., and Gledhill, A. W. (1941a). *Vet. Rec.,* **53,** 227; (1941b) *Proc. roy. Soc. Med.,* **34,** 611.
Burnet, F. M., and Timmins, C. (1937). *Brit. J. exp. path.,* **18,** 83.
Burnet, F. M., and Lush, D. (1938). *Austral. J. exp. Biol. med. Sci.,* **16,** 261.
Burnet, F. M., Beveridge, W. I. B., Bull, D. R., and Clark, E. (1942). *Med. J. Austral.,* **2,** Oct. 24, 371.
Burnet, F. M., and Bull, D. R. (1943). *Austral. J. exp. Biol. med Sci.,* **21,** 55.
Dreguss, M. (1943). *Arch. Virusforsch.,* **3,** 35. Abst. *Vet. Bull.* (1945), **15,** 113.
Glover, R. E., and Andrewes, C. H. (1941). *Proc. roy. Soc. Med.,* **34,** 615.
Gulrajani, T. S. (1951a). *J. comp. Path.,* **61,** 48; (1951b) *Ibid.,* **61,** 60.
Gulrajani, T. S., and Beveridge, W. I. B. (1951). *Ibid.,* **61,** 118.
Hjarre, A., Bakos, K., and Nordberg, B. K. (1949). *XIV International Vet. Congress, London,* Section 3 (i).
Shaw, D. R., Kenney, A. S., and Stokes, J. (Jr.). (1939). *Amer. J. med. Sci.,* **197,** 247.
Shope, R. E. (1938). *J. exp. Med.,* **67,** 729; (1941). *Ibid.,* **74,** 49.

[*Received for publication, November 20th,* 1950.]

STUDIES ON RESPIRATORY DISEASES OF PIGS

IV. Transmission of Infectious Pneumonia and its differentiation from Swine Influenza

By

T. S. Gulrajani* and W. I. B. Beveridge,

Department of Animal Pathology, University of Cambridge.

INTRODUCTION

The first three papers of this series (Gulrajani, 1951a, 1951b, 1951c) describe work on swine influenza using mostly a mouse-adapted strain (C1). When attempts were made to isolate other influenza strains from the lungs of pigs affected with what was regarded on clinical or post-mortem appearances as swine influenza, an unexpected series of negative results was obtained. Numerous inoculations of ferrets, chick embryos and mice and haemagglutination-inhibition (H.I.) tests for antibodies in inoculated pigs produced no evidence of influenza. Bacteria-free filtrates from pneumonic lungs of pigs were inoculated into pigs and produced a disease which, however, differed from swine influenza in several respects. The suspicion that the type of pneumonia we were investigating was caused by a virus other than swine influenza was strengthened by reports from Pullar (1948) in Australia, who differentiated swine influenza from a disease which he called "infectious pneumonia of pigs." Further experiments described in this paper showed that there exists in the United Kingdom a common form of pneumonia of pigs distinct from influenza and caused by a filtrable agent. It seems that this disease has sometimes been confused with swine influenza which, in our experience, is not common in this country or in Australia.

MATERIALS AND METHODS

Ferrets, mice and chick embryos. Unless otherwise stated in the text, ferrets and mice were inoculated by the intranasal route and chick embryos in the amniotic cavity. The methods employed were the same as described by Gulrajani (1951c). In most instances where chick embryos or mice were used, at least two passages were carried out before the results were considered as negative, although this is not mentioned in the text. Where the results are recorded as negative in mice it means that no pneumonia was produced. Negative results in chick embryos mean that there was no haemagglutination of r.b.c. of fowl or guinea-pig by amniotic fluid or a suspension of embryo's lungs. Negative results in ferrets mean that there was no significant rise in rectal temperature during at least 12 days observation and that the ferret developed no antibodies

* Under a grant from the Agricultural Research Council.

against C1 strain of swine influenza as shown by the modified H.I. test (Gulrajani, 1951a).

Bacteriology. As a routine, blood agar and chocolate agar plates were inoculated from four sites on the cut surface of the pig's lung and from four bronchioles, and incubated at least four days. Inoculations were also sometimes made in special medium for pleuro-pneumonia-like organisms. The bacteria were identified by colonial appearance, smears and biochemical reactions but no serological or pathogenicity tests were carried out. We did not attempt a full investigation of the part played by bacteria in pneumonia of pigs.

In order to look for a large sized virus, smears from infected lungs were examined after staining by Castaneda's and Macchiavello's methods.

Preparation of filtrates from pig lungs. A suspension of 1 in 5 of lungs in broth was made and lightly centrifuged. The supernatant fluid was diluted to make a final concentration of 1 in 50 or 1 in 100 and this suspension was passed through a Seitz clarifying pad (F.C.B.) through which 50 ml. of broth had previously been passed. The clarified suspension was then filtered through a gradocol membrane. Both filtrations were done under a negative pressure of 20 cm. to 30 cm. The filtrates were always found to be bacteriologically sterile as judged by absence of growth on blood agar plates.

Inoculation and observation of pigs. The pigs used were mostly Large Whites and were either bred at the laboratory or purchased from farms believed to be free of respiratory disease. We encountered no case of spontaneous respiratory disease in these pigs. All were used between 10 and 14 weeks of age unless otherwise stated in the text. Inoculated pigs were kept each in a separate "isolation" pen. Except where otherwise mentioned in the text, inoculations were done by the intranasal route using the technique described by Gulrajani (1951c), the dose being from 7 to 20 ml. according to the size of the pig. Before inoculation, the rectal temperature of pigs was recorded twice daily, and after inoculation three times a day for at least 12 days unless killed before then. To avoid exciting the pigs, temperatures were taken by the attendant who fed them. Pigs were killed by shooting or electrocution and were then bled out.

A serum sample was taken from each pig between the 12th to 18th day after inoculation, unless the pig was killed earlier, and tested for antibody against strain C1 of swine influenza virus by the modified H.I. test. (Gulrajani, 1951a).

Storage of pigs' lungs. Pieces of infected lungs were divided into two samples, one of which was stored at −20°C. and one in 50 per cent. buffered glycerol-distilled water at approximately 0°C. Suspensions made from stored tissue were always prepared from a mixture of the portions stored at −20°C. and 0°C.

RECTAL TEMPERATURES OF PIGS

The normal rectal temperature in pigs varies considerably, both in the same animal from time to time and from pig to pig. The temperature rises on the least excitement, so is often high and variable in pigs not used to being handled.

The temperature reactions following inoculation with virus of swine influenza have been very high and easy to interpret (Gulrajani, 1951c), but with mild attacks of infectious pneumonia following inoculation the temperature has often been difficult to interpret, in a few instances practically no elevation of temperature being present in pigs that were subsequently shown on autopsy to have pneumonia. In the later part of our work, pre-inoculation temperatures of most of the pigs were recorded for a week or longer.

Pullar (1949a) regarded a rise of 1·8°F. above the mean rectal temperature for the individual as being of pathological significance if it was recorded at more than one reading. In general, our findings are in accord with this interpretation which we have now adopted. Where pre-inoculation temperatures were not taken for at least 4 days and hence we were not able to apply Pullar's criterion, we have regarded as probably significant a temperature of more than 104·5°F., provided the animals had become thoroughly accustomed to being handled by the person taking the temperature. This interpretation of the temperature curves was, in the majority of instances, substantiated by post-mortem findings in that if a pig had a temperature of 104·5°F. after inoculation, there were nearly always lesions of pneumonia found on autopsy. However, several pigs were found to have pneumonia on post-mortem examination without having developed a temperature as high as 104·5°F. Some of these which had rather low mean normal temperatures of about 102°F. did show a rise of 1·8°F. and thus meet Pullar's criterion.

PRELIMINARY SEARCH FOR INFLUENZA

In 1943 one of us (W.I.B.B.) looked for evidence of influenza infection in pigs in Australia. Suspensions made from lungs of several pigs affected with a type of pneumonia which was macroscopically similar to swine influenza and free of bacteria were inoculated into the amniotic cavity of chick embryos, but virus was not detected. Sera of 150 pigs, mostly adults, were examined for antibody against strain S15 of swine influenza and Melb. strain of human influenza A by H.I. test or mouse neutralisation test. These sera came from several widely separated districts in Queensland, New South Wales and Victoria. Influenzal antibodies were not detected.

Our efforts in the United Kingdom during 1948 and 1949 were first directed towards isolating the virus of swine influenza in ferrets, chick embryos and mice. At this stage of the investigation pigs were not inoculated. We investigated several lots of material

which our colleagues and ourselves considered likely sources of this virus, as listed below: —

(1) Lungs of a pig which had died in an outbreak of respiratory infection in Northern Ireland, thought to be swine influenza. The lungs, preserved in glycerol, were received four days after dispatch and were bacteriologically sterile. Lungs from another pig which died in the same outbreak were received two days after dispatch.

(2) Pig lungs from a different outbreak in Northern Ireland also thought to be swine influenza, received two days after dispatch. The lungs were bacteriologically sterile.

(3) Lungs of two pigs which died in an outbreak of respiratory infection in East Anglia suspected of being swine influenza. The lungs showed extensive consolidation and were bacteriologically sterile.

(4) Lungs of a pig which died in a different outbreak in East Anglia. These also showed extensive consolidation and were bacteriologically sterile.

(5) Nasal washings of two pigs were taken at a farm near Stowmarket, Suffolk, on the 3rd day after the onset of an acute respiratory infection.

(6) Pig lungs from another outbreak of pneumonia in East Anglia. These lungs showed presence of cocci on bacteriological examination.

(7) Pig lungs from an outbreak of pneumonia in Northern Ireland received by air mail 24 hours after dispatch. These were bacteriologically sterile.

(8) Two bacteriologically sterile lungs showing areas of consolidation collected from pigs at the local slaughter-house.

Material from all the above sources was inoculated into the amniotic cavity of chick embryos and in addition four of the suspensions were inoculated into ferrets and four into mice. When bacteria were present antibiotics were added; otherwise the suspension was used untreated. In no instances was any evidence of a virus obtained. Sera from pigs recovered from one of the outbreaks were tested for antibodies against swine influenza (C1) by the modified H.I. test, but the result was negative.

TRANSMISSION OF PNEUMONIA, HITCHIN STRAINS

In view of our negative results with the inoculation of ferrets, chick embryos and mice, in our further attempts to isolate a virus we also inoculated pigs.

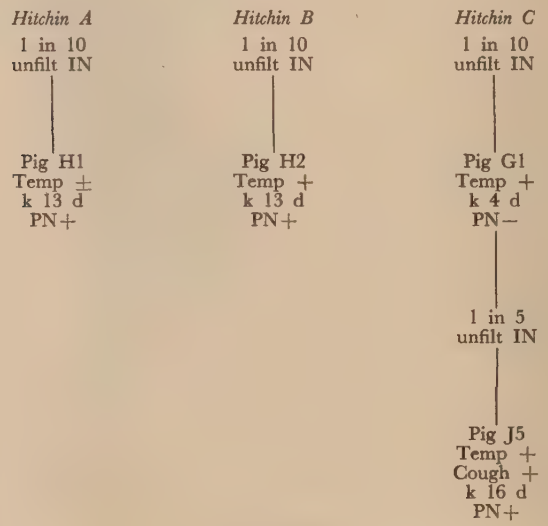
The lungs of 147 pigs slaughtered at a bacon factory were examined and tissue collected from 16 which showed pneumonia. In macroscopic appearance, the pneumonia was of the type which is often regarded in this country as due to swine influenza. The affected areas were not raised above the level of the surface of the adjoining normal lung and usually only a small proportion of the

lung was involved. The distribution of the lesions was marginal (as recently described by Pullar, 1949b, for infectious pneumonia).

On culture, six of the speciments were found to be bacteriologically sterile and only a few colonies grew from four more speciments. A heavy growth was obtained from the remaining six specimens, which were discarded. The bacteria that grew were *Bact. friedlanderi*, a Pasteurella, *Bact. coli*, Gram positive cocci and a few unidentified Gram positive bacilli.

FIGURE I

"HITCHIN" STRAINS



Key to Figures

Temp −	= no appreciable rise of temperature.
Temp +	= significant rise of temperature.
IN	= inoculated intranasally.
IV	= inoculated intravenously.
PN−	= no macroscopic pneumonia.
PN+	= macroscopic pneumonia present.
filt	= filtered.
unfilt.	= unfiltered.
k	= killed.
d	= days after inoculation.

Two pools were made, each comprising three of the sterile specimens. These were designated Hitchin A and Hitchin B. The four specimens from which only a few colonies grew were pooled and called Hitchin C.

Figure 1 is a diagrammatic representation of the pig inoculations with Hitchin strains.

Hitchin A. A suspension of 1 in 10 of the pool of lung tissue was inoculated intranasally into 11 days old pig H1, ferret 22 and

five mice. For chick embryo inoculation, a combined pool was made from Hitchin A and Hitchin B.

The results of inoculations in the ferret, chick embryos and mice were negative.

*Pig H*1. The temperature at no time rose above 103·6° F. Counts of w.b.c. were carried out daily. They fluctuated between 7,000 and 17,000 which are regarded as within normal limits. Differential counts were also within the normal range. The pig showed no symptoms, nor was antibody against strain C1 of swine influenza present in the sample of serum taken when the animal was killed on the 13th day after inoculation. The lungs showed pneumonia in both apical, left cardiac and intermediate lobes, about 20 per cent. of the lung being consolidated. The other organs appeared normal. The lungs were bacteriologically sterile.

Hitchin B. A suspension of 1 in 10 of lungs was inoculated into 11 day old pig H2 and into a group of six mice. For chick embryo inoculations, Hitchin A and B were pooled as already described.

The results of the mice and egg inoculations were negative.

*Pig H*2. During the first five days after inoculation the temperature averaged 102·2°F. and never rose above 103°F. Thereafter it reached 104° F. on five occasions. The w.b.c. counts rose from 8,000 on the day of inoculation to 20,000 on the 6th to 8th days and came down to 9,000 from the 10th day onwards. No significant changes were present in differential counts. The pig showed no symptoms, nor was antibody against strain C1 present in the sample of serum taken when the animal was killed on the 13th day after inoculation. There was macroscopic pneumonia in both apical and both cardiac lobes. A few colonies of streptococci grew on blood agar.

Hitchin C. A suspension of 1 in 10 of lungs was inoculated intranasally into pig G1, six mice and six 14-day chick embryos.

The results of inoculations of mice and chick embryos were negative.

*Pig G*1. The rectal temperature fluctuated between 103°F. and 104°F. during the first three days and then rose to 106·2°F. 76 hours after inoculation. On the 3rd day the pig coughed very much, showed inappetence and laboured respiration. On the 4th morning the temperature was down to 105·4°F., but the pig coughed much more. The w.b.c. count remained fairly constant around 20,000. Nasal washings were taken on the 4th day and inoculated into chick embryos with negative results. The pig was killed on the 4th day, and on post-mortem examination the lungs and other organs appeared normal. The lungs were bacteriologically sterile.

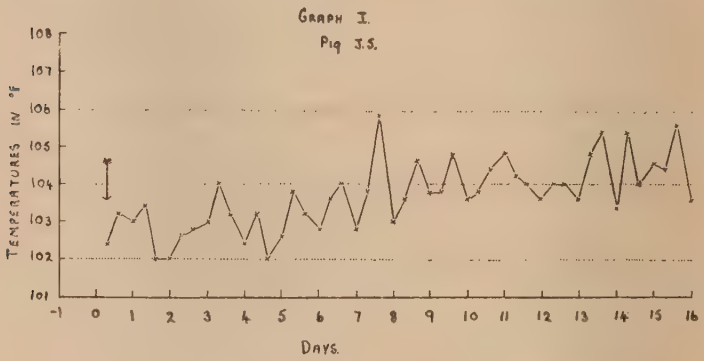
A suspension of 1 in 5 was made from pieces of the lungs of pig G1 after they had been stored for 19 days and inoculated into ferret 28 and on to the chorioallantoic membrane of eight day and eleven day chick embryos. The results were negative.

*Pig J*5. After the lungs of pig G1 had been stored for 51 days a suspension of 1 in 5 was made and inoculated intranasally into pig J5 and the inoculation repeated on two successive days. Temperature recordings are shown in Graph 1. The pig started coughing on the 13th day and this continued to the 16th day when it was killed. On the 15th

day its respiration was laboured. The lungs showed patches of pneumonia in both apical, left cardiac and intermediate lobes. The lungs were bacteriologically sterile.

Comment

Pneumonia was set up directly by inoculation of two lots of material obtained from the slaughter-house (Hitchin A and B) while the third lot (Hitchin C) produced a disease in which there was no pneumonia when the pig was killed on the 4th day but sub-inoculation from this pig set up pneumonia. Cultivable



Graph No. 1. Temperature curve of pig J5 inoculated intranasally on day 0 with strain Hitchin C.

bacteria were absent from two of the three original inocula and from the lungs of three of the four cases of disease which followed inoculation. The infective agent survived in infected lungs during 51 days' storage. Two pigs 10 and 13 weeks of age showed a significant rise in temperature, one rise starting on the 3rd day and the other on the 7th day after inoculation, but two pigs inoculated when only 11 days old did not show a marked rise even though they had pneumonia. The virus of swine influenza could not be demonstrated by inoculation of chick embryos, mice and ferrets, either with the original material or with material from the pigs inoculated at the laboratory. Antibody against strain C1 of swine influenza was not present in the post-inoculation sera of pigs and ferrets.

TRANSMISSION OF PNEUMONIA, STRAIN STORMONT

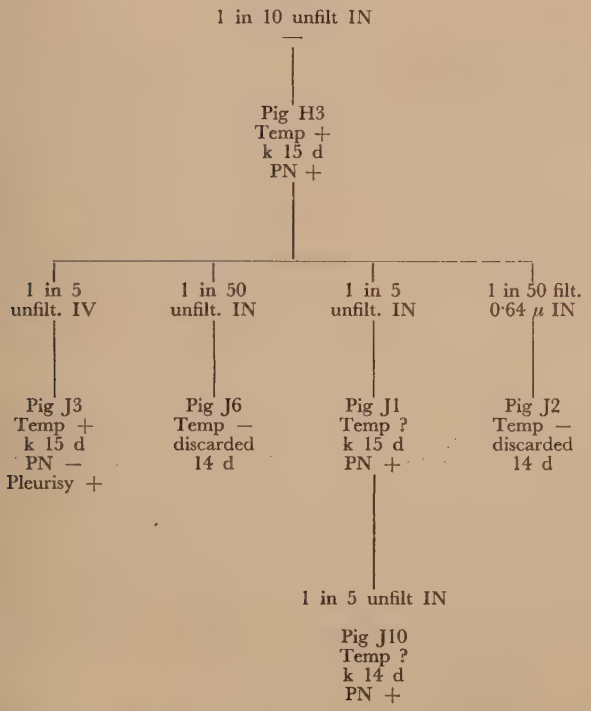
An outbreak of respiratory disease in pigs in Northern Ireland, suspected of being swine influenza, was investigated by Mr. D. Luke, M.R.C.V.S. who inoculated pigs at his laboratory with suspension of lungs of the diseased pigs. Two of the experimental pigs showed severe coughing on the 8th day after inoculation, whereupon Mr. Luke killed them and sent us their lungs in glycerol. The specimen was received within 12 hours of dispatch

and a 1 in 10 suspension prepared and inoculated into 19 days old pig H3. Mice and chick embryos were inoculated with material from a field case of this outbreak. This latter material was three days in transit. The lungs were bacteriologically sterile.

A diagrammatic representation of pig inoculations with strain Stormont is shown in Figure 2.

FIGURE II

STRAIN STORMONT



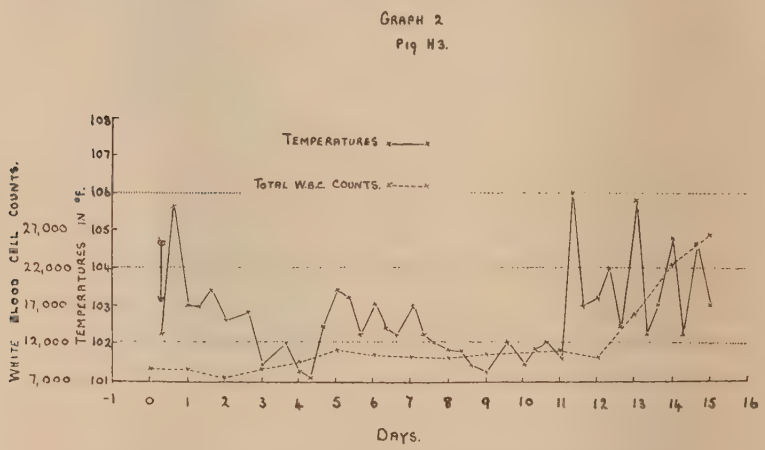
A key to the abbreviations used in this figure is shown under Figure 1.

Pig H3 received one intranasal inoculation of a 1 in 10 suspension. Temperature recordings and total w.b.c. counts of the pig are shown in Graph 2. On the 11th day, the pig showed a rise of temperature and coughed. These symptoms continued until the pig was killed on the 15th day after inoculation. The lungs showed patches of pneumonia in both apical, both cardiac and intermediate lobes. The lungs were bacteriologically sterile.

After the lungs of pig H3 had been stored 17 days, they were used for inoculating a group of four pigs belonging to the same litter, and a ferret. A suspension of 1 in 5 was made and some of

this was used to inoculate pig J1 intranasally, J3 intravenously and ferret 31 intranasally. A portion of the same suspension was diluted to make a 1 in 50 suspension; some of this was inoculated intranasally in pig J6 and some was filtered through a gradocol membrane A.P.D. 0·64 μ and inoculated intranasally into pig J2. As controls, pig J5 from the same litter was inoculated with broth and J4 left untreated. Each pig was kept in a separate pen under strict isolation.

The ferret showed no temperature or symptoms.



GRAPH No. 2. Temperature curve and total white blood cells counts of pig H3 inoculated intranasally on day 0 with strain Stormont.

Pig J3 was inoculated intravenously on 2 successive days with 10ml. of a 1 in 5 suspension. Six hours after each inoculation, the temperature rose to 105°F. but dropped to below 103°F. within 12 hours. The temperature rose to 105°F. again 53 hours after the first inoculation and an intermittent high temperature continued till the 6th day when it dropped to below 103°F. It rose again on the 11th and 12th days. When the pig was killed on the 15th day there was no pneumonia, but extensive pleurisy. The lungs were bacteriologically sterile.

Pig J6 was inoculated intranasally on two successive days with a suspension of 1 in 50 of lungs of pig H3. Pig J6 showed no significant rise of temperature or any symptoms during 14 days after inoculation, when it was discarded.

Pig J2 was inoculated intranasally on two successive days with a filtrate of a 1 in 50 suspension of lungs. The pig showed no significant rise of temperature or any symptoms during 14 days after inoculation.

Pig J1 was inoculated intranasally on two successive days with a 1 in 5 suspension. The pig showed no significant temperature or clinical symptoms and was killed on the 15th day. The lungs showed small areas of pneumonia in both apical and left cardiac lobes. The lungs were bacteriologically sterile. A suspension of these lungs was inoculated into pig J10.

Pig J5 was inoculated intranasally with sterile plain broth on two successive days, while J4 was left uninoculated. The temperatures of these pigs did not exceed 104·5°F. except for a single reading of 104·8°F. in pig J4.

Pig J10 was inoculated intranasally with a 1 in 5 suspension of lungs of pig J1. After two days a 2nd inoculum was given. The temperature showed no significant rise. The pig showed no clinical symptoms and was killed on the 14th day after inoculation. The lungs showed a moderate amount of pneumonia in both apical and cardiac lobes.

Comment

With strain Stormont, the infective agent was transmitted with bacteria-free suspensions from the original lung into pig H_3, and from the lungs of pig H_3 into pig J_1, and from this pig into pig J_{10}. Pig H_3 and pig J_1 were both killed 15 days after inoculation. It is to be noted, however, that both J_1 and J_{10} failed to show a significant rise of temperature or clinical symptoms and did not show extensive pneumonia on post-mortem. In view of this, it is not surprising that J_2 and J6 failed to show a temperature reaction to more dilute inocula; these two pigs were not killed for autopsy so it cannot be concluded that they had no pneumonia. Pig J_3, which was inoculated intravenously, showed a significant rise of temperature and pleurisy.

TRANSMISSION OF PNEUMONIA, STRAIN SELLERS

This material was obtained from an outbreak of infectious pneumonia on a farm near Cambridge. There were pigs of all ages on the farm, but acute disease was evident only in the pigs 10 to 12 weeks of age. Two of the adult boars and two gilts were said to have been "off colour", dull and depressed a week before the acute disease started. One of the sick pigs was sent to the Veterinary Investigation Centre, Cambridge, and died overnight. The lungs of this pig showed extensive consolidation of both apical, both cardiac and intermediate lobes. Diaphragmatic lobes were moderately affected. There were no other macroscopic lesions. Cultures revealed encapsulated Gram-negative bacteria, probably *Bact. friedlanderi.*

Three days after receiving the sick pig, we visited the farm and took blood from three pigs which were coughting and showing nasal discharge. It was now six days since the acute disease was first observed in the young stock. After a further 10 days, a second sample of blood was taken from these 3 pigs. The sera were titrated against strain C_1 of swine influenza, but antibody was not present.

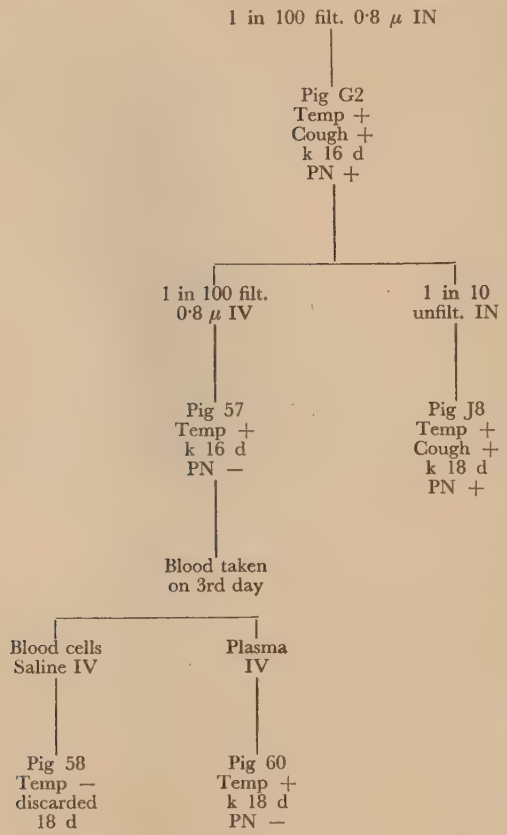
Sellers strain, passage series A

A diagramatic respresentation of pig inoculations with strain Sellers, passage series A, is shown in Figure 3.

A suspension of 1 in 100 was prepared from the lungs of the pig which died after being sent to the Laboratory and was filtered through a gradocol membrane A.P.D. 0·8 μ. The filtrate, which

FIGURE III

SELLERS STRAIN PASSAGE SERIES A.



A key to the abbreviations used in this figure is shown under Figure 1.

was bacteriologically sterile, was inoculated intranasally on two successive days into pig G2. Ferret 23 and six mice were also inoculated intranasally and chick embryos amniotically.

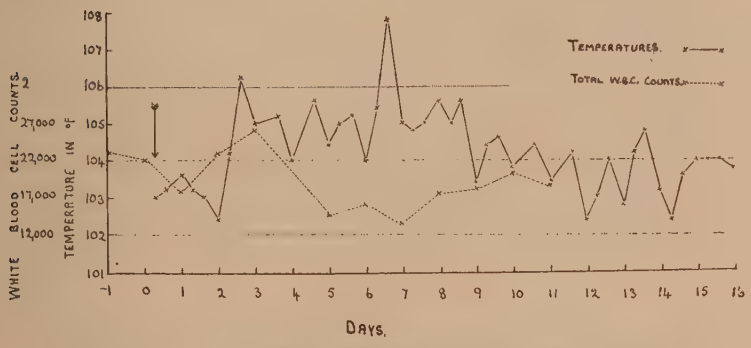
The results of the inoculations into the ferret, the mice and the chick embryos were negative, except that the ferret showed a slight rise of temperature (104°F. to 104·5°F.) on the 3rd, 4th and 8th days after inoculation.

Pig G2 developed a temperature of 106°F. at 54 hours after inoculation and the temperature remained elevated till the 9th day. (Graph 3). Daily total w.b.c. counts are also shown on Graph 3. The pig showed very laboured respiration from the 3rd to the 7th day and coughed frequently from the 9th to the 15th day. On the 9th and 10th days it was injected

subcutaneously with 30,000 units of penicillin in case the temperature reaction might be due to intercurrent bacterial infection. There were no significant changes in the differential w.b.c. counts. The pig was killed on the 16th day and showed extensive pneumonia affecting all lobes. The lungs were bacteriologically sterile.

GRAPH 3

Pig G2.



GRAPH No. 3. Temperature curve and total white blood cell counts of pig G2 inoculated intranasally on day 0 with strain Sellers.

Ferret 25 was inoculated intranasally with a 1 in 5 suspension of the lungs of pig G2. This suspension was further diluted 1 in 20 and filtered through a gradocol membrane A.P.D. 0·8 μ. This filtrate was injected intravenously in a 10 ml. dose into an 18-week-old pig, 57. After the lungs of G2 had been stored for 32 days, a suspension of 1 in 10 was inoculated intranasally into pig J8. Two days later, this pig was inoculated again with the same suspension.

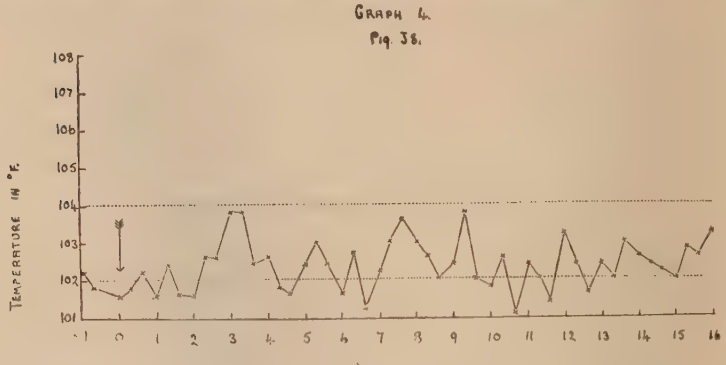
The result of the inoculation of the ferret was negative.

Pig J8 showed a significant but not marked temperature rise above the mean normal on the 3rd, 7th and 9th days and had laboured respiration on the 8th day. It coughed often on the 8th and 9th days. The temperatures of the pig are shown in Graph 4. It was killed on the 18th day and showed moderate pneumonia in the right apical, right cardiac and intermediate lobes. The lungs were bacteriologically sterile.

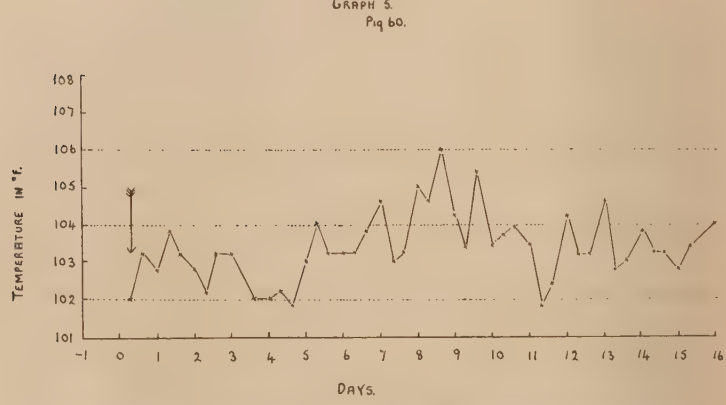
Pig 57, which had been inoculated intravenously, showed a rise of temperature to 105°F. on the 2nd and 3rd days. It was 104·8°F. on the 4th day, and 105·5°F. on the 10th day. The pig showed no other clinical symptoms and was killed on the 16th day after inoculation. The lungs and other organs appeared normal. On the 3rd day after inoculation, a sample of blood was taken and defibrinated: a portion of whole blood was inoculated intravenously into a rabbit, intraperitoneally into ferret 26 and intraperitoneally into two guinea-pigs. A portion of the blood was then centrifuged, the plasma separated and was inoculated intraperitoneally into ferret 27 and two other guinea-pigs. After the blood of pig 57 had been stored for 2 days at 4°C., pig 60 was inoculated intravenously

with plasma and pig 58 with blood cells suspended in saline. These two pigs were 19 weeks old.

Pig 60. On the 8th and 9th days after intravenous inoculation with 5 ml. of plasma, this pig showed a rise of temperature as shown in Graph 5. It showed no other symptoms and was killed on the 18th day after inoculation. Lungs and other organs appeared normal.



GRAPH No. 4. Temperature curve of pig J8 inoculated intranasally on day 0 with strain Sellers.



GRAPH No. 5. Temperature curve of pig 60 inoculated intravenously on day 0 with strain Sellers.

Pig 58. This pig showed no appreciable rise of temperature or any other abnormality after intravenous inoculation with blood cells from pig 57.

The ferrets, guinea-pigs and rabbit inoculated with material from pig 57, showed no significant rise of temperature or other abnormality. They were killed after various intervals and sub-inoculations made into animals of the same species, which in turn also failed to show evidence of infection.

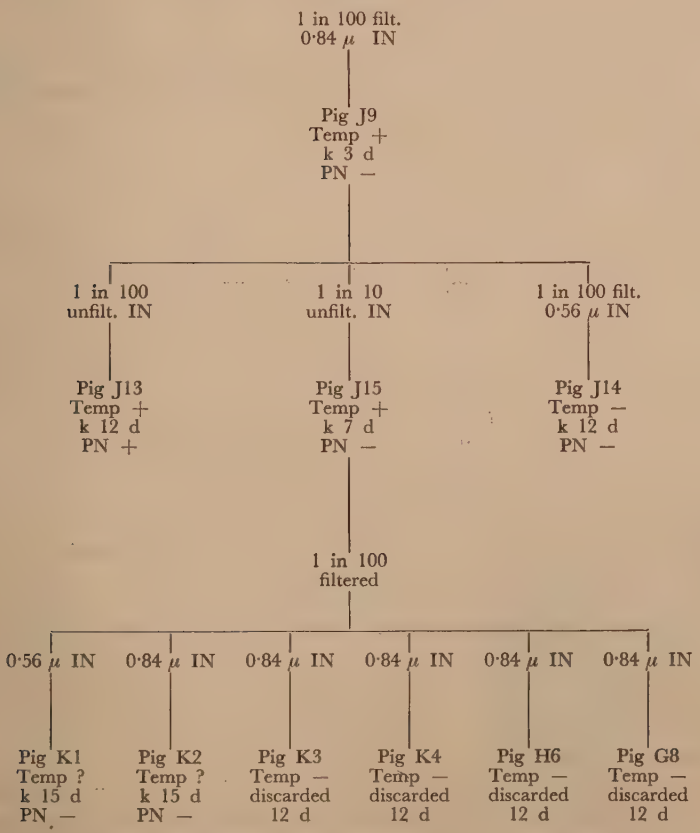
Comment

The salient points arising out of the experiments in passage series A are as follows: —

Pneumonia was reproduced in pig G2 by inoculation of a bacteria-free filtrate. The infective agent persisted in the lungs of pig G2 for 16 days after inoculation, as shown by sub-inoculation into pig J8. The disease was reproduced in pig J8 with material which had been stored for 32 days. A febrile reaction was caused in pig 57 by the intravenous inoculation, but pneumonia was not present when this pig was killed 16 days after inoculation. A febrile reaction was set up in pig 60 by intravenous inoculation of plasma from pig 57 at the height of fever, whereas no appreciable

FIGURE IV

SELLERS STRAIN PASSAGE SERIES B.



A key to the abbreviations used in this figure is shown under Figure 1.

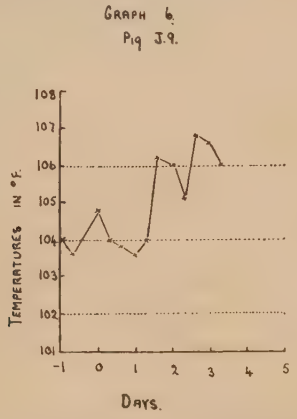
rise of temperature was produced in pig 58 inoculated intravenously with blood cells. Except for a slight rise of temperature in ferret 23 after intranasal inoculation, ferrets, rabbits and guinea-pigs showed no signs of infection after inoculation, nor did chick embryos. As noted following inoculation with the other strains, pneumonia may develop without causing a marked temperature reaction. Pig J8 showed clinical symptoms and had pneumonia when examined post-mortem, but its temperature did not reach 104°F.

Sellers strain, passage series B

The inoculations in pigs in this series are set out diagrammatically in Figure 4.

After the original lung from the field case had been stored for 55 days, a suspension of 1 in 100 was made and filtered through a gradocol membrane of A.P.D. 0·8 μ. Pig J9 was inoculated intranasally with filtrate on 2 successive days.

Pig J9 showed a rise of temperature the day after the first inoculation with the filtrate and this continued till the 3rd day (Graph 6) when it was killed. There was no pneumonia and the lungs were bacteriologically sterile.



Graph No. 6. Temperature curve of pig J9 inoculated intranasally on day 0 with strain Sellers.

After the lungs of pig J9 had been stored for eight days a 1 in 100 suspension was prepared in broth. A volume of 50 ml. was filtered through a gradocol membrane A.P.D. 0·56 μ and pig J14 was inoculated intranasally with filtrate on two successive days. Pig J13, a litter mate of J14, was similarly inoculated from the remaining portion of the suspension which had been neither clarified nor filtered.

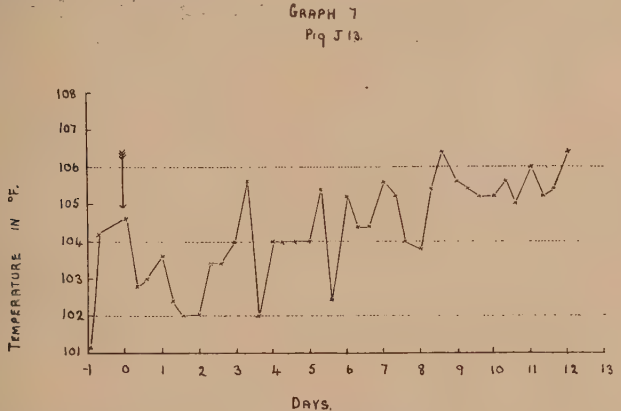
After the lungs of pig J9 had been stored for 13 days, pig J15 was inoculated intranasally with a suspension of 1 in 10 on two successive days.

Pig J14 showed no appreciable rise of temperature during the 12 days after inoculation, when it was killed. The lungs appeared normal.

Pig J13 showed a rise of temperature on the 3rd day and from the 5th to the 12th day after inoculation, when it was killed (see Graph 7). The lungs showed pneumonia in the right apical, right cardiac and intermediate lobes.

Pig J15 showed a rise of temperature on the 3rd, 5th and 6th days after inoculation and the pig was killed on the 7th day. The lungs showed no macroscopic pneumonia.

After the lungs of pig J15 had been stored for 16 days, a 1 in 100 suspension was made. A part was filtered through a gradocol membrane A.P.D. 0·56 μ, and a part through a membrane A.P.D. 0·84 μ. Pig K1 was inoculated intranasally with the former filtrate on two successive days. Pig K2 was similarly inoculated with filtrate from a gradocol membrane A.P.D. 0·84 μ.



GRAPH NO. 7. Temperature curve of pig J13 inoculated intranasally on day 0 with strain Sellers.

After the lungs of pig J15 had been stored for 27 days a suspension of 1 in 100 was made and filtered through a gradocol membrane A.P.D. 0·84 μ. Pigs K3, K4, H6 and G8 were each inoculated intranasally with filtrate on two successive days.

Pigs K1 and K2. The temperatures of these pigs rose from a normal of 103°F. to between 104·5°F. and 105°F. and remained elevated for some days. However, the pigs showed no other clinical symptoms and when killed 15 days after inoculation the lungs of both appeared normal. The result of this experiment is therefore inconclusive.

Pigs K3, K4, H6 and G8 showed no appreciable rise of temperature or any other clinical symptoms during the 12 days after inoculation.

After the original lung of the field case had been stored for 115 days, a further attempt was made to infect pigs from it by intranasal inoculation. An inconclusive result was obtained.

Comment

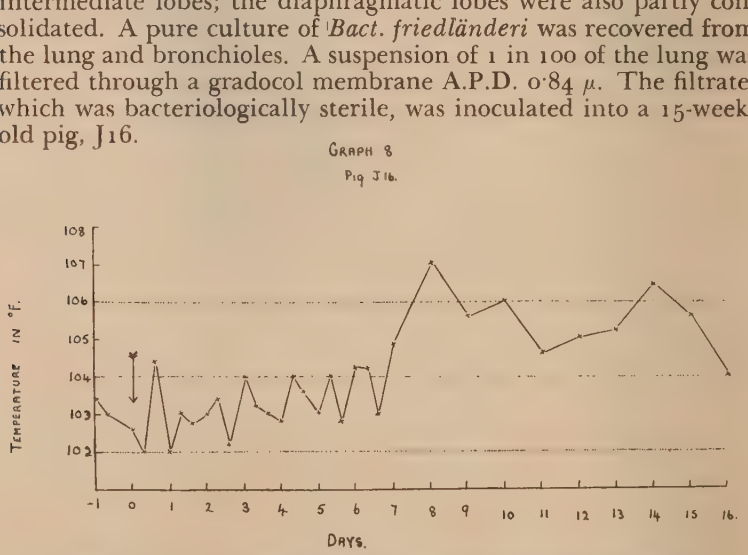
In the passage series B, the infective agent was again shown to be a filter passer, but we were not successful in maintaining it in

serial passage. The lungs of pig J9, which was killed on the 3rd day after inoculation, were shown to be infective. An inconclusive result was obtained with inoculation from the lungs of pig J15 which was killed on the 7th day after inoculation. It was shown that the infective agent survived storage for 55 days.

ATTEMPTS TO OBTAIN FURTHER STRAINS

Strain H.I.F.

An outbreak of an acute respiratory infection occurred on a farm in East Anglia and one pig died of pneumonia. The lungs showed extensive consolidation of both apical, both cardiac and intermediate lobes; the diaphragmatic lobes were also partly consolidated. A pure culture of *Bact. friedländeri* was recovered from the lung and bronchioles. A suspension of 1 in 100 of the lung was filtered through a gradocol membrane A.P.D. 0·84 μ. The filtrate, which was bacteriologically sterile, was inoculated into a 15-week-old pig, J16.



GRAPH No. 8. Temperature curve of pig J16 inoculated intranasally on day 0 with strain H.I.F.

Pig J16 was inoculated intranasally with the filtrate on two successive days. For six days after inoculation the temperature of the pig was recorded three times daily as usual but thereafter it was recorded only once daily owing to the Christmas holidays. During the first six days, the temperature fluctuated between 102°F. and 104°F., but then it rose and remained between 104·5° F. and 107°F. (Graph 8). On the 8th day, the pig's respiration was laboured and it started coughing. The symptoms continued till the pig was killed on the 17th day. It did not feed normally from the 10th day onwards. On post-mortem examination all the lobes of the lung were extensively consolidated. On bacteriological examination a heavy culture of Streptococci was recovered from the lungs. After the lungs had been stored for 30 days, a suspension of 1 in 100 was made and filtered through a gradocol membrane A.P.D. 0·84μ. This bacteriologically sterile filtrate was inoculated intranasally into pig 6654, six mice and two guinea-pigs.

Pig 6654 was inoculated intranasally with filtrate on two successive days. The pig showed no significant rise of temperature or any other clinical symptoms during 13 days of observation. When it was killed, the lungs and other organs appeared normal. The mice and guinea pigs also showed nothing abnormal.

Unsuccessful attempts

Pneumonic lungs were obtained from a pig that died in an outbreak of respiratory disease in East Anglia. Cultures of the specimens gave a heavy growth of *Bact. friedländeri*. A filtrate prepared from a one per cent. suspension was inoculated intranasally into a pig, two ferrets, two guinea-pigs and six mice. The results were negative.

Pneumonic lungs were obtained from two pigs which died in an outbreak of respiratory disease, also in East Anglia. On cultures the lungs of one pig gave a heavy growth of Pasteurella, Streptococci and *H. bronchiseptica* and were discarded. The cultures from the lungs of the other pig showed a few colonies of an unidentified bacterium from one bronchiole only. Suspension prepared from this lung was inoculated unfiltered into a pig and after filtration into another pig. The results of these inoculations were negative. Another pig from the same outbreak died in a fit. The lungs of this pig were pneumonic and were not bacteriologically sterile. A suspension of 1 in 10 was made from the brain of this pig and inoculated by the intracerebral route into a pig, two guinea-pigs and six mice. The pig and two guinea-pigs did not develop any temperature, symptoms or lesions. The mice also showed no abnormality.

Pneumonic lungs of a pig which was slaughtered at a local slaughter-house were obtained. This pig was from a different farm on which two pigs had died earlier of respiratory disease. The lungs were bacteriologically sterile. A filtrate was inoculated intranasally into a pig with negative results.

Pneumonic lungs were obtained from a seven-week-old pig which died in an outbreak of respiratory disease at the farm from which we had earlier obtained strain Sellers. The lungs were bacteriologically sterile. A suspension was inoculated intranasally on two successive days into a pig with negative results.

DISCUSSION

We have transmitted in pigs six strains of an infectious agent causing pneumonia. The resemblances between the diseases produced by each strain and the circumstances of the origin of the strains have led us to assume tentatively that we have been dealing with a single disease entity. Working on this assumption, the main points arising out of the investigation may be summarised as follows.

1. The disease has been produced three times by intranasal inoculation of filtrate which had been passed through gradocol membrane A.P.D. 0·8 μ, and a further 10 times by suspensions of lung tissue which were free of bacteria. The lung lesions thus produced were usually themselves free of bacteria, though in some instances bacteria were present.

2. In the pigs in which pneumonia was set up, a significant rise in temperature sometimes occurred on the 2nd, 3rd, 4th and 5th day after inoculation. When this early temperature reaction occurred, the temperature usually remained elevated for at least four days. In other cases there was no rise of temperature till the 8th, 9th, 10th or 11th day. The temperature rise was seldom very marked and several pigs did not develop a temperature that could be regarded as significant by any standards, even though they were found on autopsy to have pneumonia.

3. Macroscopic pneumonia was not present in the lungs of three pigs which showed a definite temperature reaction and symptoms and were killed on the 3rd, 4th and 7th days after inoculation. Pneumonia was present in several of the pigs which were killed between the 12th and 16th days after inoculation. It was of the type which is prevalent in pigs seen in slaughter-houses in this country and in Australia, that is marginal in distribution and not raised above the level of the normal lung.

4. A rise of temperature has been caused in two pigs by the intravenous inoculation with lung filtrate, but pneumonia was not present in pigs thus inoculated. Extensive pleurisy was present in one of them. Plasma from the blood of a pig which had been inoculated intravenously with lung filtrate and showed a significant rise of temperature, caused a rise of temperature in a further pig inoculated intravenously.

5. The disease has been transmitted from lungs of two pigs killed on the 3rd and 4th day after inoculation. It is not clear whether the disease has been transmitted from lungs of a pig killed on the 7th day. The disease has, however, been produced from lungs of pigs killed on the 12th to 16th days, but not consistently.

6. An attempt to maintain one strain by serial passage (Sellers strain passage series B) failed after two transfers and another strain (H.I.F.) did not survive beyond the first passage. Serial passage was not attempted with the other four strains, one of which was carried for three transfers.

7. In a single experiment, the disease was not produced by a filtrate through a gradocol membrane A.P.D. 0·56 μ, while the same suspension used unclarified and unfiltered produced disease.

8. The disease has been produced after infected lung had been stored at $-20°$ C., and in glycerol at 0° C., for 32, 51 and 55 days. It is, however, not known which of the two methods of storage is better.

9. There was a rise of temperature of doubtful significance in

only three of the many ferrets inoculated intranasally, and there was no other evidence that the infection could be established in ferrets.

10. There was no evidence of the multiplication of the infective agent in the amniotic cavity or on the chorio-allantoic membrane of chick embryos, nor in mice after intranasal inoculation. A "grand pool" of passaged embryonic fluids and tissues was inoculated intranasally into a pig, but it showed no reaction.

11. Serological tests (H.I.) for swine influenza (strain C1) on pigs which developed the disease and on inoculated ferrets were consistently negative. Pairs of pre- and post-inoculation sera from strains Sellers, Stormont, H.I.F. and Hitchin C were also tested by the conglutinating-complement absorption test for antibodies against swine influenza virus (strain C1) by the technique described by one of us (Gulrajani, 1951d). Antibody was not present in these sera.

Only six strains of infective agent were isolated out of ten attempts made.

These experimental results leave little room for doubt that we are dealing with a specific infectious pneumonia caused by a virus, but we continue to seek confirmation of this view. For the present, we shall refer to this disease as "infectious pneumonia of pigs", partly because it is non-committal as to cause or relationship to certain other pneumonias which have been described in pigs and partly because it closely resembles the disease recently described by Pullar (1948) under that name.

We differentiate this disease from swine influenza on the following grounds: —

Clinical picture. Infectious pneumonia develops more slowly than does swine influenza. In the former, the temperature does not rise until at least 48 hours after inoculation and sometimes not until over a week, whereas in swine influenza, the temperature is usually distinctly elevated 24 hours after inoculation and in uncomplicated cases it falls in the next day or so. With infectious pneumonia the pigs often appear practically normal clinically and continue to eat even though they have a temperature and pneumonia, especially during the first week or so after inoculation. With swine influenza, the pigs appear distinctly ill during the first two days and lose their appetite, but within a week they have recovered clinically, unless the disease becomes complicated by secondary bacterial infection.

Post-mortem picture. The type of pneumonia seen in the two diseases is similar macroscopically. With swine influenza, pneumonia may be present as early as the 3rd day after inoculation and is well marked in pigs killed on the 7th day, whereas with infectious pneumonia we have not seen pneumonia in pigs killed from three to seven days after inoculation, the earliest pneumonia seen in our series being in a pig killed 12 days after inoculation.

Isolation of virus. In a large number of attempts we have failed to detect virus in infectious pneumonia by using the methods by which influenza viruses can readily be isolated.

Serological evidence. Tests for antibody against C1 swine influenza have been consistently negative in pigs infected with infectious pneumonia. (It should be noted, however, that these serological tests are rather strain specific and may fail to detect antibody against a heterologous strain of swine influenza.)

Persistence of infectious agent in lungs. We have demonstrated the continued presence of the causal agent of infectious pneumonia for more than two weeks after inoculation and the general impression obtained in the field and slaughter-house is that it persists for long periods; on the other hand, one of us has not been able to recover the C1 strain of swine influenza 10 days after inoculation (Gulrajani, 1951c).

Routes of infection and location of infectious agent in the host. Whereas the virus of swine influenza is present only in the lungs and associated lymph glands and infection can be set up only by inoculation into the respiratory tract, we have some experimental evidence (admittedly not conclusive) that the infectious agent of infectious pneumonia may be present in the circulation and may be transmitted by intravenous inoculation.

A cross-immunity experiment between these two diseases was planned on more than one occasion but unfortunately the causal agent of infectious pneumonia was found to have lost its infectivity when it was required. The tendency for this infectious agent to "peter out" on passage and the difficulty of consistently reproducing the disease has hampered our investigations in several directions. However, a cross-immunity test is not essential to our investigation and would show little more than have our serological tests, for they measure the protective antibody against influenza. The absence of cross-immunity between the two diseases would not itself prove that infectious pneumonia is not caused by a virus of the influenza species, because it is known that some strains of influenza virus fail to protect against each other.

The study of the respiratory infections of pigs continues in our laboratory, there obviously being need for much further work. However, we feel that the recognition of infectious pneumonia as being distinct from swine influenza is a useful advance. There is little doubt that much confusion has been caused in the past by failure to distinguish between these two diseases. It seems to us probable that infectious pneumonia as here described is a very prevalent disease, at least in the United Kingdom.

We feel that to attempt to relate all other investigators' reports on respiratory diseases of pigs to our findings without a long discussion may only tend to perpetuate the confusion already existing in this field. For this reason we omit reference to that work for the present and propose to review the position fully later.

It may be of interest to draw attention to the resemblances between infectious pneumonia of swine and a form of atypical pneumonia in man. In both diseases the symptoms are much

milder than one would expect from the nature and extent of the lesions, both are long-lasting infections and both are due to an infectious agent which can not be demonstrated by usual techniques used in bacterial or virus diseases.

SUMMARY

Attempts were made to isolate new strains of swine influenza viruses from outbreaks of respiratory diseases in pigs. The material used was obtained from outbreaks in East Anglia and in Northern Ireland. In addition, the lungs of pigs showing consolidation were obtained from a bacon factory and some lungs were collected at local slaughter houses. A good many of the lungs used were bacteriologically sterile. Sometimes pigs alone were inoculated, but in most cases ferrets, mice and developing chick embryos were also inoculated. Transmission experiments with bacteria-free filtrates of lung suspensions indicated the presence of a filterable agent in several specimens, but the clinical symptoms in pigs thus inoculated differed considerably from those produced by inoculation of swine influenza virus, and no antibodies were produced against the latter virus. No virus was isolated in chick embryos, ferrets or mice.

These observations suggest that swine influenza is not prevalent at present in the United Kingdom and that the form of infectious pneumonia which is most common is due to a virus different from swine influenza. This disease appears to be widespread and needs further study.

ACKNOWLEDGMENTS

We are grateful to Dr. H. G. Lamont, Messrs. K. C. Sellers, H. I. Field, D. Luke and J B. Busby who have kindly supplied specimens used in these investigations.

REFERENCES

Gulrajani, T. S. (1951a). *J. comp. Path.*, **61,** 48; (1951b). *Ibid.,* 60; (1951c). *Ibid.,* 101; (1951d). In press.

Pullar, E. M. (1948). *Austral. vet. J.,* **24,** 320; (1949b). *Ibid.,* **25,** 53; (1949a). *Brit. vet. J.,* **105,** 437.

[*Received for publication, December 28th,* 1950.]

CANINE CHRONIC NEPHRITIS*

I. Observations on the Pathology of the Kidney.

By

H. Platt.

Department of Experimental Pathology, Guy's Hospital Medical School, London.

INTRODUCTION

The discovery of the organism *Leptospira canicola* by Klarenbeek and Schüffner (1933) and its subsequent incrimination as an important causal agent in the etiology of canine acute nephritis, have stimulated much interest in recent years in the renal diseases of dogs. However, observations upon the histopathology of nephritis of the dog have been much less numerous than in the human subject. The pathological changes in the kidneys of dogs affected with various stages and types of nephritis have been studied by M'Fadyean (1929) and by Bloom (1939; 1941). More recently, MacIntyre and Stuart (1948) have reported on the frequency of agglutinating antibodies against *Leptospira canicola* among street dogs and have described the natural history of the disease to which this organism frequently gives rise. They consider that acute leptospiral nephritis is an important antecedent factor in the pathogenesis of chronic nephritis.

The present account is devoted to a survey of the naked-eye and microscopical changes which have been observed among 25 dogs affected with varying degrees of chronic nephritis. All these animals, with the exception of three, were males. No information was available regarding the nature of the etiological agents responsible for the initial renal damage. The kidneys of eight animals which had died or had been destroyed within two to three weeks of the onset of acute nephritis were also examined. They provided important information regarding the mode of development of the lesions which were present in the chronic stage of the disease.

METHODS

Apart from examination of the brain, complete autopsies were carried out on all the animals whose kidneys were studied. Material for histological examination was obtained as soon after death as possible, but in some instances a period of some three or four hours elapsed before this was done. On this account little attention has been paid to the cytological changes in the tubular epithelia, since the kidney is very susceptible to post-mortem autolytic changes. All tissues were fixed in 10 per cent. neutral formol-saline solution, dehydrated in alcohol and embedded in paraffin

* This paper formed a part of a thesis presented to Liverpool University for the degree of M.V.Sc.

wax. Sections were cut at a thickness of 6 microns and were stained as a routine with haematoxylin and eosin. Other staining methods which were frequently employed in selected instances were those of van Gieson, von Kossa, and Weigert (for elastic tissue). Pieces of tissue were prepared by Levaditi's method for the demonstration of leptospira. Frozen sections were also prepared and stained by Scharlach R. for the detection of fats. Any lipoid material observed was examined by polarised light using crossed Nichol prisms.

ACUTE NEPHRITIS

Before proceeding to the changes observed in chronic nephritis, a brief description will be given of the pathology of canine acute nephritis based upon the findings in the eight cases which have been examined.

Gross appearances

Acute inflammation of the kidneys was usually accompanied by some degree of swelling of these organs and in the more severely affected cases this change was very pronounced. On palpation, the kidneys were softer than usual. Cross sections revealed intense cortical pallor due to the very dense infiltration with inflammatory cells in this region. The capsules stripped off without difficulty leaving a smooth surface often flecked with haemmorhagic mottlings.

Microscopical changes

The changes in all the kidneys studied were very similar and they will therefore be described together.

The predominant histological characteristic in every case examined was an intense, widespread, tubular and interstitial nephritis. In the most severe cases, large areas of the renal cortex were almost obliterated by dense accumulations of inflammatory cells. The changes were most intense in the deeper parts of the cortex and particularly in the regions near the boundary zone, but they also extended diffusely into the more superficial parts of the cortex. In the worst affected areas both the tubules and the glomeruli had been destroyed. Elsewhere, in regions where the inflammation was less severe, the tubular epithelium showed marked swelling and other degenerative changes; in places, tubular epithelial cells were undergoing mitosis. The glomeruli in these less severely affected areas showed no apparent change.

In the inflamed areas, the great majority of the cells present were histiocytes and other mononuclear elements, but there were also small groups of polymorphonuclear leutocytes in places. The convoluted tubules often contained small masses of necrotic cells and cell debris, and similar casts were found in the medullary tubules. Changes in the renal medulla were much less striking than in the cortex and were restricted principally to the region near the boundary zone. Elsewhere, only small focal patches of inflamation were present.

A careful search for leptospira was made in sections prepared by Levaditi's method, but no unequivocal evidence of the presence of the organisms could be obtained.

CHRONIC NEPHRITIS

Gross Appearances

The degree of structural alteration in the kidney that is apparent to the naked eye depends largely upon the severity of the changes that are present. It is worthy of note, however, that a serious degree of renal disease may be present without very pronounced alteration in the gross appearance of the kidneys. In general, severe chronic nephritis was accompanied by a reduction in size and in the most advanced cases this change was present in an extreme degree. In such instances the kidneys were often very pale in colour owing to extensive replacement of the parenchyma by comparatively avascular fibrous tissue. As a rule, the capsules stripped readily or with occasional adhesions, leaving a surface which was granular or irregular according to the amount and distribution of the fibrous tissue lying in the cortex beneath. In the more seriously affected kidneys, the surface was often speckled with small white points, which represented groups of tubules protruding slightly from the surface of the organ and separated from each other by scar tissue. There was some increased firmness on cutting and when the kidney was examined in section, it could be seen that, in the more severe cases, there was a pronounced narrowing of the cortex. Cystic tubules were sometimes visible to the naked-eye in the cortex, but were much more frequent in the medulla which in consequence sometimes assumed a polycystic appearance. The deposition of new fibrous tissue was apparent in both the cortex and the medulla, but in the former it showed a particular predilection for the cortical side of the boundary zone where it could often be clearly seen as a well-marked pale line.

Microscopical changes

The histological changes may be classified under the following main headings: (*a*) changes representing exaggerations of normal senescent processes; (*b*) degenerative changes in the glomerular and tubular basement membranes; (*c*) fibrotic and compensatory changes; (*d*) inflammatory changes; (*e*) metastatic calcification of the kidneys.

Hypertensive changes in canine nephritis will be described in a subsequent publication (Platt, 1951).

Changes Representing Exaggerations of Normal Senescent Processes

Pericapsular fibrosis

This is a change sometimes seen in kidneys which in other respects appear to be perfectly normal, but in such instances it is usually present only to a slight degree. In some cases of chronic nephritis, however, the change may be present to a grossly

exaggerated extent with the formation of dense collars of fibrous tissue around the glomerular capsules (Figure 1). Any distensibility normally possessed by the glomerular capsule must be very seriously reduced. It seems possible that this change might be a reaction to excessive distension of the glomerular capsules, in the course of the compensatory hypertrophy which the glomeruli frequently undergo in chronic nephritis. The change is often associated with hyalinisation of the glomerular tufts, but it is not clear whether there is any association between the two events.

Hyalinisation of glomeruli in chronic nephritis

It has been observed by Moritz and Hayman (1934) that, in human kidneys, obliteration of glomeruli by hyalinisation takes place continuously throughout life and appears to be a normal phenomenon. They also found that the hyalinised glomeruli finally disappeared without leaving any trace of their former presence. It seems very probable that the same holds true for the dog. In view of the large proportion of hyalinised glomeruli which were observed in the kidneys examined in the present study, an opportunity presented itself for the detailed examination of the process in the form in which it occurs in chronic nephritis.

In canine nephritis, the process of hyalinisation appears to begin in the root of the glomerular tuft with the formation of an amorphous, faintly eosinophil material which tends to obscure the capillaries between which it lies. The process then spreads to the periphery of the tuft and is accompanied, even when hyalinisation is incomplete, by a marked reduction in the size of the tuft and in its vascularity. Sometimes, the hyalinised tuft, often surrounded by a distended capsular space, undergoes no further change while the parietal layer of Bowman's capsule, with its thickened and wrinkled basement membrane, collapses around it. From this point onwards, the two tend to become fused into a homogeneous mass of faintly eosinophil material. In other cases, however, a second process also takes place. The swelling and puckering of the capsular basement membrane causes wrinkles and pockets to appear in the normally even contour of Bowman's capsule whose cavity often becomes filled with a fine web of collagen. Hyalinising glomeruli are frequently closely invested with a network of fine collagen fibres arising from the inner surface of the glomerular capsule. The line of demarcation between the hyalinised tuft and the collagenous ingrowths from the capsule rapidly becomes indistinguishable. The hyalinised tuft then becomes progressively fibrosed in the same manner as before, shrinks, becomes less eosinophilic and finally disappears.

It is of interest to examine the state of the tubule after hyalinisation of its respective glomerulus. It is possible that a proportion of tubules may become aglomerular nephrons, since McNider (1933) has shown that this type of nephron is to be found in the kidneys of dogs in which the acute renal damage, produced by the injection

of uranium nitrate, is succeeded by fibrosis. In some cases at least, the tubule degenerates and this process has been studied in serial sections from the kidney of an animal with chronic nephritis. It was occasionally possible to trace the site of origin and early part of the tubule from the old hyalinised glomerular capsule, and when this was done it was seen that a complete desquamation of the lining epithelium had taken place and that there was gross swelling of the tubular basement membrane. The original lumen of the tubule was almost indistinguishable. The grossly swollen basement membrane showed basophil staining affinities and retained approximately the shape of the original tubule. It had an amorphous granular texture (Figure 2). In the later stages of the process, it gradually stained less deeply and finally became merged with the surrounding fibrous tissue. Often in the neighbourhood of old hyalinised glomeruli, no trace of their former tubules could be found in the local fibrous tissue.

There appears to be very little information on the exact nature or cause of glomerular hyalinisation. In chronic nephritis, the process is greatly exaggerated and the following observations give some idea of the extent to which glomerular hyalinisation takes place in this condition. The numbers of functional and hyalinised glomeruli were counted in ten low power (2/3″) microscope fields (×80).

TABLE I

PROPORTIONS OF FUNCTIONAL AND HYALINISED GLOMERULI IN TEN MICROSCOPICAL FIELDS IN NORMAL AND NEPHRITIC KIDNEYS.

Case No.	*Age*	*Functional glomeruli*	*Hyalinised glomeruli*
A. *Normal Kidneys*			
1	4 months.	37	0
8	9 years.	47	0
15	11 years.	37	0
16	2½ years.	31	0
B. *Chronic Nephritis.*			
4	12 years.	37	23
13	12 „	18	10
23	10 „	27	22
24	8 „	11	12
29	9 „	10	38
31	7 „	13	10
32	4 „	30	30
34	3 „	18	11
38	10 „	11	7
40	11 „	47	6

From these figures it is evident that in normal kidneys hyalinisation probably only affects a small proportion of the glomerular tufts at any given time. In chronic nephritis, however, the proportion of hyalinised glomeruli is very greatly increased.

Degenerative Changes in the Glomerular and Tubular Basement Membranes

Degenerative changes in the basement membranes of the glomerular capsules have already been mentioned in connection with the process of glomerular hyalinisation. In chronic nephritis, particularly in grossly fibrosed kidneys, the change was often very extensive and severe. It affected the basement membranes of both the tubules and the glomerular capsules, and the change was often present in tubules whose epithelium was intact and apparently functional. The earliest changes in the basement membranes were seen during acute nephritis when they showed intense basophil staining and stood out as a fine sharply defined line (Figure 3). On closer examination at this stage, it could be seen that the membrane had a granular and particulate appearance. No calcium was detectable by Von Kossa's method. In the later stages of nephritis, basophil staining of the membrane was no longer evident, but marked swelling was present which developed until eventually the basement membrane appeared as a pale staining, thick band.

Fibrotic and Compensatory Changes

The development and distribution of scar tissue

A process such as acute nephritis, which involves widespread destruction of the glomeruli and tubules, is likely to be succeeded by the development of granulation tissue and scarring when healing occurs. Regeneration of the specialised tubular epithelium is only likely to take place on a limited scale and in regions of less intense inflammation where the lining cells of the tubules have been only partly destroyed.

Extensive scarring of the kidneys was present in all the cases of chronic nephritis. It was most marked in the neighbourhood of the boundary zone, but large areas of scar tissue were often seen in the more superficial parts of the renal cortex. Near the boundary zone there was often destruction of many of the juxtámedullary glomeruli and their tubules with replacement by a thick layer of dense fibrous tissue, up to 2mm. in width, from which bands of scar tissue radiated out into the overlying cortex. Lying in the scar tissue were distorted, shrunken and other abnormal types of tubule. The disposition of the scar tissue coincided fairly well with those areas which were most intensely inflamed in acute nephritis.

Changes in the glomeruli

Well marked compensatory hypertrophy could readily be seen in the glomeruli in chronic nephritis and it was often associated with gross distension of the capsular spaces. The number of functional glomeruli was usually very much reduced, due partly to widespread hyalinisation and probably also to destruction of glomeruli during an antecedent attack of acute nephritis. Many of the functional glomeruli, particularly those lying in and near

areas of fibrosis, showed the presence of hyaline eosinophil protein in the capsular space, suggesting that there was some increase in the permeability of the glomerular capilliaries. Adhesions of the glomerular tufts to the inside of the capsules were occasionally found, but endothelial or epithelial proliferation of the glomerular tufts were never seen in this series of cases.

Changes in the tubules

The appearance of the convoluted tubules varied widely in different parts of the cortex of the same kidney. In some areas, groups of tubules lined by epithelium with eosinophil cytoplasm showed no apparent change from the normal. Elsewhere, the tubules were often shrunken and atrophic, and the cytoplasm of their epithelial cells showed basophil staining affinities. As a rule, many dilated tubules were present and in some of the more grossly distended nephrons the epithelium was extremely flattened. The tubules often contained hyaline eosinophil protein. Variable quantities of isotropic fat were often found in the convoluted tubular epithelium, but this is a normal feature of the canine kidney. In the late stages of chronic nephritis, a frequent and very striking feature was the development of an epithelial metaplasia in the collecting tubules, particularly in the medullary part of their course. Although it was often associated with tubular dilatation this did not appear to be essential for the development of metaplasia since the change was seen in tubules which at the time of examination showed no sign of distension. The metaplastic epithelium consisted of up to six or seven layers of closely packed cells with hyperchromatic nuclei and showed a very close resemblance to the transitional epithelium of the renal pelvis with which it was in continuity. The change sometimes affected the collecting tubules lying in the cortex, but this was less frequent. Many of the cells, comprising this metaplastic type of epithelium, showed a curious cytoplasmic vacuolation. Isotropic neutral fat was present in the epithelial cells in the form of small droplets, but it was not clear whether this would account for all the vacuolation which was seen in paraffin sections, since in the most severely affected cells the cytoplasm was almost completely replaced by a large vacuole. The possibility that this vacuolated appearance was due to an artefact could not be entirely excluded.

Inflammatory Changes

Many of the kidneys in the present series have been the site of varying degrees of cellular infiltration, particularly in the areas of scarring and fibrosis. In the earlier stages of chronic nephritis, plasma cells, fibroblasts and cells which were apparently lymphocytes were the principal types present. In those kidneys which were examined at a later stage of the disease, where widespread formation of dense collagen had taken place, cellular infiltration was less

evident. In the small groups of cells, which were to be found at this stage of nephritis, plasma cells were infrequent. Small round cells, apparently lymphocytes, were present in addition to other cells which were distinguishable from them by their more extensive, grey staining cytoplasm and their larger size. The precise nature of the latter could not be decided.

In a single animal, focal acute inflammation was present in the form of a small abcess in the renal cortex composed of polymorphonuclear leucocytes surrounded by a wide dense cuff of histiocytes. No gram-positive organisms were present in the lesion. The dog was known to have suffered from acute leptospiral nephritis a year previously. Renal fibrosis in this instance was relatively slight.

In several cases of severe chronic nephritis, small groups of polymorphonuclear leucocytes and necrotic cell debris were present in occasional collecting tubules. No foci of acute inflammation could be found but the possibility that such lesions were present, but were located in regions of the kidney outside that taken for histological examination, could not be excluded. The collecting tubules in which they occurred were all very dilated and showed epithelial metaplasia.

Metastatic Calcification

Deposition of calcium in the kidneys was frequently present in chronic nephritis. The change affected particularly the basement membranes of the convoluted tubules, but calcium was sometimes present in the walls of the glomerular capsules and in small aggregations in the interstitial tissue (Fig. 4). It was only occasionally found free in the tubular lumen and was then in a particulate form; casts of calcium in the tubules was not found. In the medulla, calcification was almost absent, even in cases where the cortex was severely affected. No calculi were found in the renal pelvis or ureters. In one case of severe chronic nephritis, in which dense calcification was present in the basement membranes of the convoluted tubules, a fine web of collagen fibres had formed between the calcified basement membrane and the tubular epithelium. This change was present in a number of tubules near the surface of the cortex. In one animal, calcification of the basement membrane took the form of localised, spherical laminated deposits which displaced the overlying tubular epithelium towards the centre of the lumen.

DISCUSSION

The chronic renal changes described in this paper have been designated throughout as those of a chronic nephritis. It is possible that in some cases the term "renal fibrosis" would be preferable. This was particularly true of the more severely affected kidneys where, in many instances, the renal changes were of long standing.

In many of these cases inflammatory changes were greatly overshadowed by those of repair and fibrosis. However, the term chronic nephritis, in its usual rather loose connotation, has been retained in this paper since it is almost universally used in connection with renal changes of this type.

The morbid histological lesions observed in the cases of acute nephritis conformed closely with the descriptions of acute leptospiral nephritis recorded by Bloom (1941). The distribution of the lesions and the widespread destruction of the renal parenchyma were such that reparative changes would probably have led to the type of fibrosed and scarred kidneys which were found in the dogs with chronic nephritis. By the time that this stage has been reached, however, histological methods alone, in the absence of other data, provide little information regarding the nature of the destructive or inflammatory renal changes which may have preceded the development of scar tissue, and it is possible that the antecedent renal damage in the present series of cases was not always of the same aetiological type.

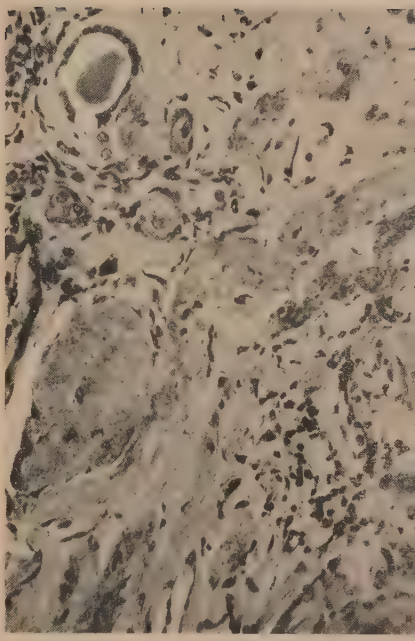
The high proportion of hyalinised glomeruli which are present in chronic nephritis is of some interest from a functional point of view. Its significance in this respect will depend upon whether the nephron whose glomerulus has undergone hyalinisation was still a functioning unit at the time when the process commenced. If hyalinisation of the glomerulus merely represents a sequel to the destruction or disuse of its respective tubule, the disappearance of the glomerulus from an already functionless nephron would have little further effect. On the other hand, if hyalinisation of the glomerulus occurs before any serious tubular change, the progressive loss of functioning nephrons as a result of the hyalinisation of their glomerular tufts must be an important factor in producing a progressive deterioration in renal functional capacity. To some extent this would be compensated for by the hypertrophy of the remaining glomeruli, but since these are possibly already enlarged to compensate for the glomeruli destroyed during antecedent acute renal damage, the effect could not be other than to increase the functional demands upon nephrons which are already overworked. Another possibility is that after glomerular hyalinisation, a proportion of the tubules survive and function as aglomerular nephrons. This seems not unlikely in view of their occurrence in the scarred kidneys which develop following uranium nitrate poisoning in dogs. (McNider, 1933.)

SUMMARY

The morbid anatomy and histopathology of eight cases of acute nephritis and 25 cases of chronic nephritis are described in dogs affected with the naturally occurring forms of these diseases.

Fig. 1

Fig. 2



Figuré 1. A glomerulus showing severe pericapsular fibrosis. H. & E. × 500.

Figure 2. A hyalinised glomerulus and the grossly swollen basement membrane of its tubule. H. & E. × 500.

Fig. 3

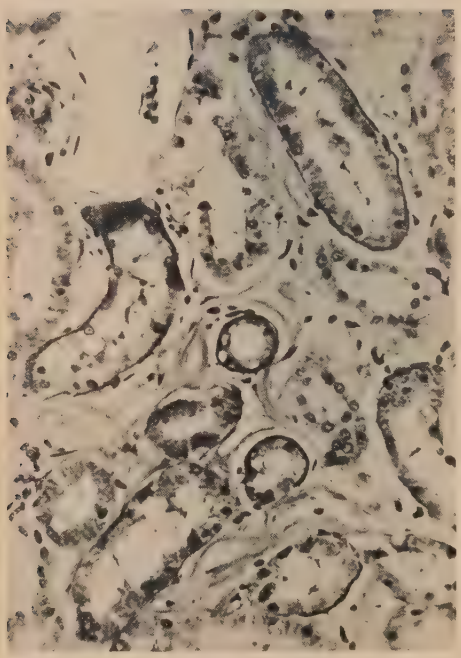


Fig. 4

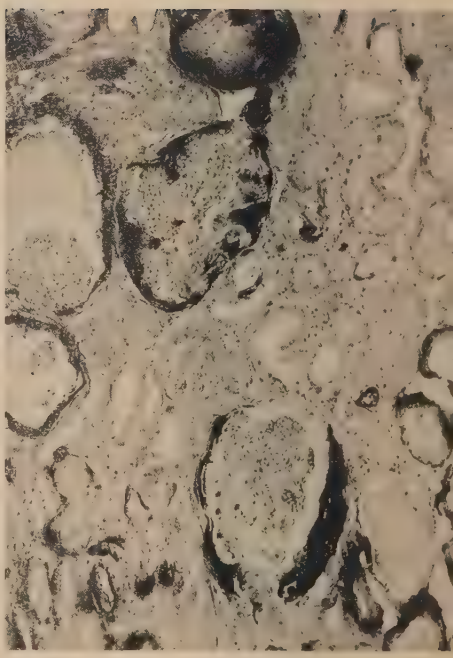


Figure 3. Early basement membrane change in the tubules of the renal cortex. Note the similarity to calcification of the basement membranes. H. & E. × 250.

Figure 4. Metastatic calcification of the kidney. Note the extensive involvement of the basement membranes and the glomerular capsules. von Kossa × 104.

ACKNOWLEDGMENTS

I should like to express my deep appreciation of the advice and help which I have received from Professor G. Payling Wright during the period in which the work upon which this paper and others in the series are based was carried out. I should also like to thank him for his criticism of them. I am indebted to Messrs. J. F. C. Wilder, R. S. Morgan and L. Till for technical assistance; and to the Agricultural Research Council and the Animal Health Trust for grants.

REFERENCES

Bloom, F. (1939). *Arch. Path.*, **28,** 236; (1941). *Cornell Vet.*, **31,** 266.
Klarenbeek, A., and Schüffner, W. (1933). *Ned. Tijdschr.*, **77,** 4271.
MacNider, W. de B. (1933). *Proc. Soc. exp. Biol. Med.*, **31,** 293.
M'Fadyean, J. (1929). *J. comp. Path.*, **42,** 141 & 231.
McIntyre, W. I. M. and Stuart, R. D. (1949). *Vet. Rec.*, **61,** 411.
Moritz, A. R., and Hayman, J. M. (1934). *Amer. J. Path.*, **10,** 505.
Paltt, H. (1951). In press.

[*Received for publication, December 16th,* 1951.]

A NOTE ON A NEW METHOD FOR PREPARING A POTENT ANTI-CHAUVOEI VACCINE

By

M. Sterne, P. Thorold, and J. R. Scheuber

Onderstepoort Laboratory, Pretoria, and Kabete Laboratory, Nairobi

INTRODUCTION

McEwen (1926) grew *Clostridium chauvoei* in broth which was separated from meat particles by a parchment diffusion cell. He obtained abundant growth, free from particulate media constituents. After formolization, his cultures immunized guinea pigs and sheep against substantial doses of virulent *Cl. chauvoei*. Sterne and Wentzel (1950) produced very potent toxoids from *Clostridium botulinum* Types C and D grown in intussuscepted cellophane tubes (Wentzel and Sterne, 1949) immersed in nutrient medium. This method differed from McEwen's in that the clostridia were not grown in broth, but in saline into which the media constituents dialysed. Thus the antigens eventually used contained no protein except that derived from the bacterial growth. The adaptation of this technique to the large-scale production of anti-chauvoei vaccine is decribed in the present note.

METHODS

Apparatus

The apparatus (Fig. 1) was, generally, as described for the production of botulinum toxins. The outer container is a pyrex tube (A) 4ft. 6 ins. long by 9 ins. in diameter. An intussuscepted cellophane tube, Visking's $5\frac{1}{2}$ins. diameter standard stretch sausage casing, stretched to 7ins. diameter (B) contains 3 litres of saline (C); it is immersed in 30 litres of liver-meat broth containing 1 per cent. commercial cane sugar (D). Arrangements are made for inoculating and harvesting, and for adding alkali and sugar during growth. The culture vessel and its contents are sterilized at 15lbs. pressure for four hours.

Growth and Detoxication

The dialysate in the annular space in the cellophane tube was inoculated with a twenty-four-hour culture of *Cl. chauvoei*. Alkali and sugar were added whenever growth flagged. After eight days, 2·5 litres of a heavy suspension of clostridia were harvested from the cellophane tube. This suspension was allowed to settle in the refrigerator overnight, and the fairly clear supernatant was then decanted and clarified by filtration through a medium grade of filteraid. The filtrate was incubated at 37°C. with 0·3 per cent. formalin for 48 hours. The dense suspension which remained after decanting the supernatant was incubated with 0·6 per cent. formalin for 48 hours. Neither the formolized filtrate nor the

sediment caused significant reactions when injected into guinea pigs or sheep in 5 ml. amounts. The filtrate and sediment were then mixed (a procedure suggested by J. C. MacSween in a personal communication) and this mixture constituted the con-

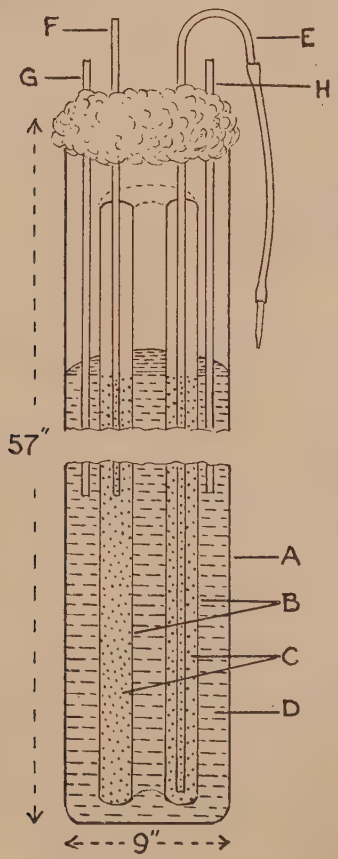


Fig. 1.

A = Pyrex culture vessel. B = Intussuscepted cellophane tube.
C = Culture in saline. D = Medium. E = Harvesting siphon.
F = Inoculating tube. G and H = Tubes to alkali and sugar reservoirs.

centrated vaccine. Dilutions of the vaccine were adsorbed on to aluminium phosphate (Holt, 1947) and tested on sheep. The results of an experiment are set out in Table I.

An inspection of Table I shows that 1 ml. of a 1/50 dilution of the anaculture protected sheep against 100 lethal doses of unactivated *Cl. chauvoei* culture. At this dilution, the 2·5 litres

RESULTS

TABLE I

No. of Sheep.	*Type of vaccine.*	*Dose in ml.*	* *Dilution of concentrated vac.*	*No. of MLD used for immunity test after 3 weeks.*	*Results.*
2	Formolized filtrate ...	2	1/10	200	LL
1		2	1/100	200	*
2	Formolized sediment ...	2	1/10	200	LL
1		2	1/100	200	L
2	Anaculture	1	1/10	100	LL
2	(sediment added	1	1/25	100	LL
2	back to filtrate) ...	1	1/50	100	LL
2	Nil (controls)	—	—	2	* *
2	Nil (controls)	—	—	1	* *

* = Diluted in $AlPO_4$ 15mgm./ml. (Holt, 1947).
* * = Died. L = Lived.

harvested from the one culture vessel yielded more than 100,000 doses of vaccine. The reactions of the sheep to the vaccine were limited to barely detectable transient thickenings of the skin of the thigh without any lameness. The number of animals in this experiment was small, but the size of the test dose gives the results considerable significance.

Further experiments were carried out to test the effects of different media, and of different methods of adsorption on the effectiveness of the "dialysate" vaccines. Moreover, some lots of sheep were immunized with formol filtrates and some with anaculture. Although these tests have not been completed, all the results, both good and bad, have been tabulated to show the effect of dosage and dilution on the efficiency of the "dialysate" vaccines (Table II).

The summary in this table shows that "dialysate" vaccines are very effective antigens. Most of the deaths occurred in groups immunized with formolized filtrates, a result which is an additional confirmation of the work of McEwen (1926), Henderson (1932), Mason (1936a, 1936b) and Roberts (1946), on the superiority of anacultures for immunisation against *Cl. chauvoei*. There was no indication that toxoid adsorbed on aluminium phosphate was superior to toxoid precipitated with alum in the conventional way. The last experiments shown in Table II were done with ratios of saline to medium of 1 : 3, and yielded eight litres of culture per flask. One ml. of a 1/25 dilution of a formolized filtrate of this culture immunized sheep against a large test dose. This meant a yield of 200,000 doses from one culture flask. No anaculture made from this culture was tested.

TABLE II

No. of Sheep.	*Dose vaccine in ml.*	* *Dilution of vaccine.*	*Ratio of dialysate to medium.*	*No. of MLD in test given 3 weeks later.*	*Results*
2	5	1/10	1/10	80—100	LL
4	5	1/10	1/10	50—80	LLLL
2	5	1/50	1/10	30—40	LL
5	5	1/50	1/10	80—100	LLL **
2	5	1/100	1/10	30—40	LL
14	2	1/10	1/10	100—200	LLLLLLLLLLLLL
11	2	1/20	1/10	50—80	LLLLLLLLLL
4	2	1/40	1/10	50—80	LLLL
2	2	1/50	1/10	30—40	LL
3	2	1/50	1/10	80—100	L **
2	2	1/100	1/10	30—40	LL
2	2	1/100	1/10	200	L * **
4	1	1/10	1/10	100	LL ** **
2	1	1/25	1/10	100	LL
2	1	1/50	1/10	100	LL
6	1	1/10	1/3	50—100	LLLLL
6	1	1/25	1/3	50—100	LLLL * **
2	1	1/50	1/3	100	** **

* = Some lots $AlPO_4$ adsorbed, and some alum-precipitated.

** = Deaths in these lots in animals immunized with filtrate, not anaculture.

DISCUSSION

The preliminary work reported in this paper showed that "dialysate" toxoids of *Cl. chauvoei* were far more potent antigens than toxoids prepared in the conventional way. Although the results were not nearly so striking as those obtained with *Cl. botulinum* Types C and D, they were sufficiently promising to warrant the trial of the method for the preparation of chauvoei vaccine. A yield of 100,000 doses per culture vessel compares very favourably with the usual harvest of about 2,000 doses. The convenience of being able to use 1 ml. doses in place of the larger doses usually employed is an added advantage. We are confident that further work will result in considerably improved yields.

SUMMARY

A method has been described for producing potent anti-chauvoei vaccine in intussuscepted cellophane tubes. Yields of 100,000 to 200,000 doses per culture vessel were obtained.

Doses of 1 ml. of the diluted vaccines were effective in eliciting a high degree of immunity in sheep.

The reactions to vaccination were in all cases negligible.

ACKNOWLEDGMENTS

Part of this work was done at the Onderstepoort Laboratory, Pretoria (M.S and J. R. S.), and part at the Kabete Laboratory, Nairobi (P.T.). We thank the Director of Veterinary Services, South Africa, and the Director of Veterinary Services, Kenya, for permission to publish.

REFERENCES

Henderson, D. W. (1932). *Brit. J. exp. Path.*, **13,** 421.
Holt, L. B. (1947). *Lancet,* **252,** 282.
MacSween, J. C. *Personal communication.*
Mason, J. H. (1936a). *Onderstepoort J. vet. Sc. and anim. Ind.,* **7,** 143; (1936b). *Ibid.,* 433.
McEwen, A. D. (1926). *J. comp. Path.,* **39,** 253.
Roberts, R. S. (1946). *Ibid.,* **56,** 128.
Sterne, M., and Wentzel, L. M. (1950). *J. Immunol.,* **65,** 175.
Wentzel, L. M., and Sterne, M. (1949). *Science,* **110,** 259.

[*Received for publication, January 15th,* 1951]

LEPTOSPIRA ICTEROHAEMORRHAGIAE INFECTION IN PIGS

By

D. I. Nisbet

Veterinary Investigation Department
The Edinburgh and East of Scotland College of Agriculture

INTRODUCTION

Infection in pigs with organisms of the genus Leptospira has not previously been recorded in Great Britain. This paper describes the investigations made in two cases of jaundice in young pigs occurring on separate farms in the South-east of Scotland.

In Great Britain the only domestic animals from which leptospirae have been isolated are the dog and the calf. Okell, Dalling and Pugh (1925) first isolated *L. icterohaemorrhagiae* from dogs, and Field (1949) and Field and Sellers (1950) have reported the isolation of a strain of the same organism from a calf.

In other countries *L. icterohaemorrhagiae* infection in pigs has been recorded by Sander (1935), Klarenbeek and Winsser (1937), Hougardy (1938) and Collier (1948).

According to Broom (1949), the leptospira most commonly found in pigs is *L. pomona,* first isolated in Australia from a human case of Queensland Fever by Clayton, Derrick and Cilento (1937) and later found in pigs by Johnson (1939). Buchanan (1927) mentions attendants in piggeries as being exposed to considerable risk of infection with *L. icterohaemorrhagiae* on account of the usual rat infestation of the premises.

DESCRIPTION OF OUTBREAKS

Clinical History

Case 1. On this farm pigbreeding had only been commenced recently and was carried out on a large scale. About 130 young pigs from 3 to 8 weeks old were running with the sows in a long building, partitioned into four courts by stone walls. The buildings were infested with rats. The disease occurred in three of the four courts. The feed store was situated at one end of the line of courts and the largest number of cases was found in the court nearest to this store, the court farthest away being that in which no cases occurred. Affected young pigs were seen to be listless and standing apart from the others with their ears drooping. Jaundice usually occurred about 24 hours later, and was first noted in the ears where it was most obviously seen. Two days later the animals became prostrate and died soon afterwards. Death was invariable once jaundice had appeared. About 10 deaths had occurred before the spread of the disease was checked by penicillin therapy (see Treatment).

An affected pig three weeks old, which had not been treated with penicillin, was received for examination. It was in a prostrate condition with pyrexia and showing extreme jaundice of the skin and all visible mucous membranes. The animal was destroyed and a post-mortem examination made.

Case 2. Sporadic cases of a disease characterised by jaundice had occurred in pigs on this farm for five years prior to this investigation with intervals of up to a year between cases. An interesting feature was that affected pigs were invariably in litters from bought-in gilts and never in litters from the homebred gilts. The course of the disease was from 7 to 14 days after the pigs had been noticed to be ill, and there was usually a fatal termination although a few had recovered. Two months previous to this investigation two cases had occurred which had been treated with penicillin and both had recovered. This piggery was also infested with rats.

The pig received for examination was the only one affected of a four-weeks-old litter of ten from a bought-in gilt. It was alive when examined and vigorous in its movements and reactions. The skin and visible mucous membranes were very jaundiced and its temperature was 103·5° F. The animal was destroyed and a post-mortem examination made.

Treatment

On the farm from which case No. 1 was received, penicillin therapy was instituted as soon as a tentative diagnosis of leptospiral infection had been made, with encouraging results. As soon as a young pig was noticed to be listless and standing apart from the others, 100,000 units of sodium penicillin were given intramuscularly, the dose being repeated on the two following days. About 20 pigs were treated in this manner, with only one death which occurred in a pig already showing jaundice before treatment. Similarly on the second farm two pigs showing jaundice were successfully treated.

Penicillin would thus appear to be highly effective in the treatment of this condition in pigs.

Post-mortem Findings

The changes were similar in both cases. Jaundice extended throughout all the body tissues, together with numerous small haemorrhages in the lungs, intestinal walls, and retroperitoneal tissues. In Case 1 the spleen also showed haemorrhages and was enlarged and congested.

ISOLATION OF THE ORGANISM

The possibility of a leptospiral infection was evident from the clinical symptoms and post-mortem findings. Accordingly urine and heartblood from Case 1, and liver and kidney from Case 2 were examined under the darkground microscope. The results, however, were negative.

Cultural Methods

In both cases cultures, made from liver, kidney, heartblood and spleen in the modification of Fletcher's medium recommended by Stuart (1946), proved negative for leptospirae after incubation for 14 days at 30°C. Blood agar plate cultures from the same organs and the bone marrow incubated aerobically at 37°C. remained sterile.

Biological Methods

Case 1. Small pieces of liver and kidney were ground up finely and suspended in broth. This suspension was centrifuged at 1,000 r.p.m. for 1 minute to throw down gross particles and 1 cc. was inoculated subcutaneously into each of two guinea pigs. Five days later both guinea pigs appeared healthy, but ten days after inoculation one died with jaundice evident in the ears, pads of the feet and the conjunctiva. Post-mortem examination made within a few minutes of death showed generalised jaundice with numerous haemorrhages giving the typical "butterfly wing" appearance in the lungs and haemorrhages in the perirenal fat. The pericardial sac contained about 1 cc. of blood-tinged fluid. A wet preparation made from the kidney and examined by dark-ground illumination showed leptospirae. A similar examination of urine and heartblood was negative. Blood agar cultures from liver and heartblood incubated aerobically remained sterile.

The other guinea pig died the following day. Post-mortem examination several hours after death showed similar lesions. No leptospiral organisms could be demonstrated in the liver or the kidney. No further investigation was made in this case.

Case 2. A liver and kidney suspension was inoculated into a guinea pig, which remained apparently healthy and was destroyed six days after inoculation. No lesions were found on post-mortem examination, no leptospirae were demonstrated by darkground examination of the liver and kidney, and an agglutination test on the serum against *L. icterohaemorrhagiae* antigen was negative. No leptospirae were recovered in cultures from heartblood and from liver in Fletcher's medium and in Noguchi's medium. A suspension of 1 cc. of liver and kidney from this guinea pig was inoculated subcutaneously into each of two guinea pigs. Eight days later one appeared sick and showed jaundice. It was destroyed and post-mortem examination showed the typical picture of *L. icterohaemorrhagiae* infection, *i.e.* haemorrhages into all organs and yellow, pinhead-sized necrotic foci on the surface of the liver. Darkground examination of liver and kidney preparations showed numerous leptospirae in both organs. The remaining guinea pig was destroyed the following day. Similar lesions were found and leptospirae were also present in the liver and kidney.

Cultures made from the heartblood of these two guinea pigs in the modified Fletcher's medium showed a good growth of leptospirae after 15 days incubation at 30°C. The strain has been

maintained by subcultures made at intervals of about two weeks. Agglutinin absorption tests on the cultures have identified the organism as *L. icterohaemorrhagiae*. Under darkground illumination the organisms showed the typical morphology of the genus Leptospira.

HISTOLOGY

Sections of pig liver and kidney stained by Levaditi's method showed spirochaetal organisms in both organs. In the kidney these were seen within the cells of the convoluted tubules, only a few being observed to lie within the lumina. Sections stained by haematoxylin and eosin were also examined. In the liver the pathological changes were of a diffuse nature. Many hepatic cells were necrotic and, of those surviving, the majority showed some degree of degeneration. In the kidney most of the glomeruli showed a thickening of Bowman's capsule, those in which this change was most marked also showing disruption of the glomerulus. The normal tubular architecture was greatly altered, individual tubules often being difficult to discern and the tubular epithelial cells in areas throughout the cortex appearing to be conglomerated, forming irregular masses with few distinct lumina. Cellular casts, consisting of a few polymorphs and desquamated tubular epithelial cells, were present in a number of the convoluted tubules. In the medulla eosinophilic material occupied some of the tubules, and in the interstitial tissue there were focal accumulations of lymphocytes and macrophages. The picture was thus one of tubular nephritis.

SEROLOGICAL FINDINGS

About six months after the original outbreak from which Case 1 was received, during which time no further cases had occurred, a visit was made to the farm. The rat population had been very largely exterminated shortly after the outbreak had occurred. On this occasion samples of blood were taken from nine pigs which, if not actually recovered animals, had been in the same group as the affected pigs. Also by chance one rat was caught. The sera from these bloods were subjected to agglutination tests using *L. icterohaemorrhagiae* and *L. pomona* antigens. The results were as follows: —

	L. icterohaemorrhagiae	*L. pomona*
Pig 1 ...	Negative	Negative
Pig 2 ...	1 : 30	Negative
Pig 3 ...	1 : 30	Negative
Pig 4 ...	1: 30	Negative
Pig 5 ...	Negative	Negative
Pig 6 ...	1 : 30	Negative
Pig 7 ...	1 : 100	Negative
Pig 8 ...	1 : 30	Negative
Pig 9 ...	1 : 30	Negative
Rat	Negative	...

Definite conclusions cannot be drawn from these figures, in view of the fact that records of antibody titres against *L. icterohaemorrhagiae* in normal pig sera are not available.

In Case 2 sera from the original pig and four of the guinea pigs used for the passage of the infective agent were subjected to agglutination tests using *L. icterohaemorrhagiae* and *L. canicola* antigens. The results were as follows: —

	Jaundice	*Titre against L. icterohaemorrhagiae*	*Titre against L. canicola*
Pig	+	1 : 3000	1 : 100
Guinea pig 1st Passage (destroyed at 6 days) ...	—	—	—
Guinea pig 2nd Passage (destroyed at 8 days) ...	+	—	—
Guinea pig 2nd Passage (destroyed at 9 days) ...	+	1 : 10	—
Guinea pig 4th Passage (destroyed at 38 days) ...	—	1 : 1000	—

DISCUSSION

Difficulty was experienced in the isolation of *L. icterohaemorrhagiae* from pigs, all cultures remaining sterile in both outbreaks and only biological methods proving successful. Even so, in Case 2 the first passage guinea pig was apparently healthy when killed six days after inoculation, and on post-mortem all efforts to demonstrate leptospirae were negative. Only in the second passage was the typical picture of leptospiral infection seen. A guinea pig in the fourth passage also remained seemingly healthy until destroyed 38 days after inoculation. On post-mortem examination yellow, depressed, pinhead necrotic foci were observed in the liver and the serum agglutinated *L. icterohaemorrhagiae* antigen to a titre of 1 : 1000, indicating that the animal had become a carrier. These two instances illustrate the well-known fact that the virulence of *L. icterohaemorrhagiae* for the guinea pig is very variable.

Negative titres have been obtained in the agglutination test on the sera of two guinea pigs, used in passaging the organism, one of which seemed healthy and the other was clinically affected. It would therefore appear that a negative titre in the guinea pig, at least up to eight days after inoculation with presumed infective material, is no indication of the absence of infection.

With regard to culturing the organism it is important to allow a sufficient period of incubation before cultures are declared negative and discarded. In Case 2 the primary culture was not positive until the twelfth day. The organism had to be subcultured at intervals of a week to ten days initially, as by then motility had decreased and some degenerate forms were seen.

The source of infection in the original cases is a matter for conjecture, but probably was provided by the rats which infested

both piggeries. In Case 1 the rats appear to have come from the food-store and contaminated the foodtroughs with urine. The organism then gained entrance to the pigs either by penetration of intact skin or mucous membrane or via skin injuries.

No human case of leptospiral infection has been known to occur on the two farms mentioned. Nevertheless, the possibility of the transmission of the disease to man through contact with urine from affected pigs and by handling material at post-mortem examination is a real one, and appropriate precautions should be taken by attendants and others handling such animals.

SUMMARY

Two cases of leptospiral jaundice in young pigs, occurring in separate piggeries, are recorded.

An account of the disease on both premises is given together with the post-mortem findings.

The isolation of *L. icterohaemorrhagiae* by guinea pig inoculation from both pigs and its subsequent culture in one case are described. The serum of nine pigs from one farm was subjected to agglutination tests against *L. icterohaemorrhagiae* antigen.

The virulence of *L. icterohaemorrhagiae* for the guinea pig is discussed, and some difficulties encountered in obtaining and maintaining a growth of the organism in vitro are mentioned.

The results of penicillin therapy in other cases on the same farms are given.

ACKNOWLEDGMENTS

My thanks are due to Mr. J. T. Stamp for his continued interest in this work and to Mr. J. A. Watt for helpful advice.

I am indebted to Mr. W. I. M. McIntyre of the Royal (Dick) Veterinary College for performing the agglutination tests and for much valuable advice. I wish also to thank Dr. J. C. Broom of the Wellcome Laboratories of Tropical Medicine for identifying the organism.

Case-histories and material were supplied by the kindness of Mr. N. W. Keith.

REFERENCES

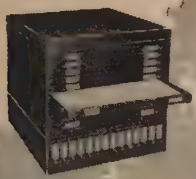
Broom, J. C. (1949). *Vet. Rec.*, **61,** 44, 711.
Buchanan, G. (1927). *Spec. Rep. Ser. Med. Res. Counc.*, No. 113.
Clayton, G. E. B., Derrick, E. H., and Cilento, R. (1937). *Med. J. Austr.*, **1,** 647.
Collier, W. A. (1948). *Schweiz. med. Wschr.*, **78,** 508.
Field, H. I. (1949). *Proc. roy. Soc. Med.*, **42,** 719.
Field, H. I., and Sellers, K. C. (1950). *Vet. Rec.*, **62,** 311.
Hougardy, A. (1938). *Brux. Med.*, **18,** 1163.
Johnson, D. W. (1939). *Proc. 6th Pacific Sci. Cong.*, **5,** 331.
Klarenbeek, A., and Winsser, J. (1937). *Dtsch. tierärztl. Wschr.*, **45,** 434.
Okell, C. C., Dalling, T., and Pugh, L. P. (1925). *Vet. J.*, **81,** 3.
Sander, F. (1935). *Arch. Hyg.*, **113,** 279.
Stuart, R. D. (1946). *J. Path. Bact.*, **58,** 343.

After this article had been submitted, a paper was published by Field, H. I., and Sellers, K. C. (1951). *Vet. Rec.*, **63,** 78, also describing this condition.

[*Received for publication, January 20th,* 1951]

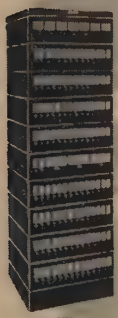
more than a mere laboratory instrument . . . . . .

The constant-temperature Water Bath for floating paraffin sections

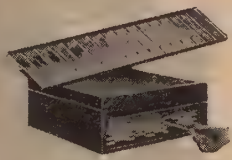


The Block File stores paraffin blocks with utmost economy of space

The Slide Dryer dries slides in seven minutes

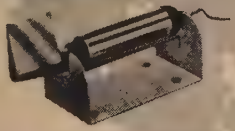


The Slide-files for all types of slides and transparencies



The Laboratory Slide Trays and Cabinet

The standardized reagents for tissue processing and staining



The electric Paraffin Knife for cutting, blocking and mounting tissues

The Mobile Refrigerator for gathering and storing tissues

an integrated system for every phase of histologic tissue preparation and reference

Autotechnicon

Trade Mark Registered U S Patent Off

automatic tissue processing by any histologic technique

The Autotechnicon System, as employed in a typical hospital working day, is interestingly discussed in an informative brochure. A copy will be sent on request to . . .

In thousands of hospitals, the world over, the benefits of the Autotechnicon System of mechanized tissue-processing procedures are being demonstrated daily. Production is improved, time saved, efficiency heightened, labor conserved. The Autotechnicon System is unique . . . the outgrowth of decades of intimate study of, and experience with, the technical problems of the pathologic laboratory.

Address in U.K.: 7 LYTTON GARDENS, WALLINGTON, SURREY

A woodcut by Thomas Bewick (1753-1829) of the Chillingham Bull. Reproduced from a print in the Hornby Collection, Liverpool Public Libraries, through the courtesy of the City Librarian.

In penicillin therapy **'DISTAQUAINE'** brand preparations of procaine penicillin offer the following advantages :

- ★ *Aqueous, containing neither oil nor wax*
- ★ *Easy to prepare and administer*
- ★ *Least possible pain on injection*
- ★ *Effective blood levels up to 24 hours following administration*
- ★ *Dry syringe unnecessary*
- ★ *Equipment easily cleaned after use*

'DISTAQUAINE' *SUSPENSION*, **vials of 10 ml. (300,000 units per ml.)**
'DISTAQUAINE' G. vials of 300,000, 900,000 and 3,000,000 units.
'DISTAQUAINE' *FORTIFIED*, **vials of 400,000 and 1,200,000 units.**

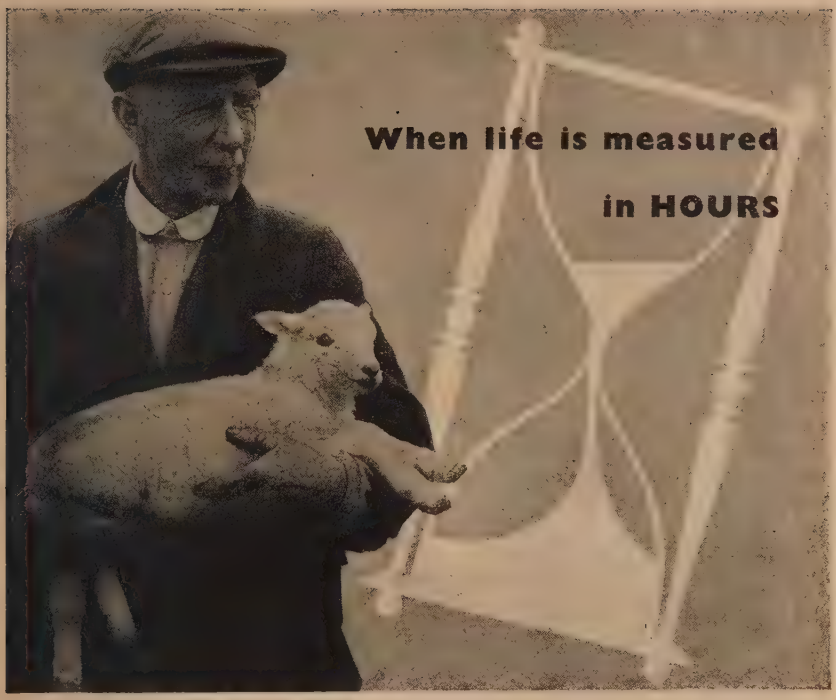
available from

Allen & Hanburys Ltd., British Drug Houses Ltd., Burroughs Wellcome & Co., Evans Medical Supplies Ltd., Imperial Chemical (Pharmaceuticals) Ltd., Pharmaceutical Specialities (May & Baker) Ltd.

'Distaquaine', a trade mark, is the property of the manufacturers

THE DISTILLERS COMPANY (BIOCHEMICALS) LIMITED

SPEKE **LIVERPOOL**



An injection of 'Wellcome' Concentrated Lamb Dysentery Serum not later than twelve hours after birth offers the best means of protecting new-born lambs against lamb dysentery. This serum, a 2 c.c. dose of which is as efficient as a 5 c.c. dose of the unconcentrated product, also provides an immunity against pulpy kidney disease, sufficient to protect the lamb during the first three to four weeks of life. This latter immunity is not permanent and should be reinforced at four weeks of age by an injection of 'Wellcome' Pulpy Kidney Serum. 'Wellcome' Concentrated Lamb Dysentery Serum is issued in containers of 50 c.c. (25 doses) at 28s. 0d., *subject to professional discount.*

'Wellcome' BRAND

Concentrated **Lamb Dysentery Serum**

PREPARED AT THE WELLCOME RESEARCH LABORATORIES

SUPPLIED BY

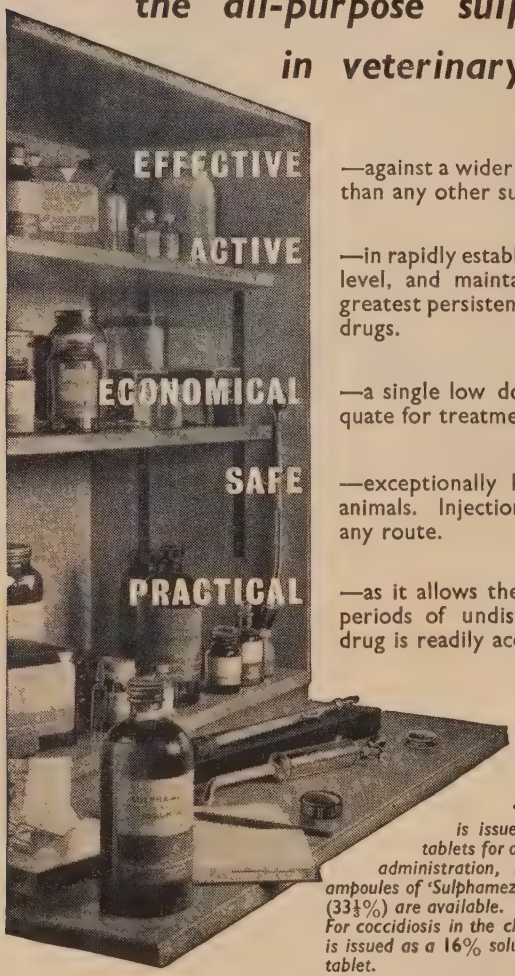
BURROUGHS WELLCOME & CO. *(THE WELLCOME FOUNDATION LTD.)*

183-193, EUSTON ROAD, LONDON, N.W.1, Tel. EUSton 4477 AND 18, MERRION SQUARE, DUBLIN, Tel. DUBLIN 64460

'SULPHAMEZATHINE'

(Sulphadimidine B.P.C.) TRADE MARK

the all-purpose sulphonamide in veterinary practice



—against a wider range of infections than any other sulphonamide.

—in rapidly establishing a high blood level, and maintaining it with the greatest persistence of all the sulpha drugs.

—a single low dosage daily is adequate for treatment.

—exceptionally low toxicity in all animals. Injection can be made by any route.

—as it allows the sick animal long periods of undisturbed rest. The drug is readily accepted in food.

'SULPHAMEZATHINE' is issued as a powder, and in tablets for oral use. For parenteral administration, multi-dose bottles and ampoules of 'Sulphamezathine' Sodium Solution (33⅓%) are available.
For coccidiosis in the chick 'Sulphamezathine' is issued as a 16% solution, and as a solution tablet.

Literature and further information available, on request, from your nearest I.C.I. Sales Office—London, Bristol, Birmingham, Manchester, Glasgow, Edinburgh, Belfast and Dublin.

IMPERIAL CHEMICAL (PHARMACEUTICALS) LIMITED

A subsidiary company of Imperial Chemical Industries Limited

WILMSLOW **MANCHESTER**

ICI

Ph.76/2

antihistaminic *therapy in veterinary practice*

trade mark **'ANTHISAN'** *brand* mepyramine maleate is a compound possessing powerful antihistaminic properties and is of low toxicity. Its use is indicated in the treatment of many allergic conditions and anaphylactic reactions. Included in the conditions where a gratifying response has been regularly obtained are such as laminitis and allergic skin conditions, including urticaria and moist eczema.
A growing volume of evidence suggests that, in addition to normal antibacterial treatment, 'Anthisan' has an important place in the treatment of acute septic metritis.

detailed information on the indications and dosage of 'Anthisan' will be supplied on request.

'Anthisan' is supplied as follows :—
Solution (5 *per cent.*) Boxes of 10 x 10 c.c. ampoules
Solution (2.5 *per cent.*) Boxes of 10 x 2 c.c. ampoules
Cream (2 *per cent.*) Collapsible tubes of 1 oz. and 4 oz.

VETERINARY PRODUCTS M&B

TO THE PROFESSION ONLY

manufactured by **MAY & BAKER LTD** V48457

distributors

PHARMACEUTICAL SPECIALITIES (MAY & BAKER) LTD., DAGENHAM

for epidural and regional anaesthesia

trade mark
'PLANOCAINE'
brand **procaine hydrochloride**

an anaesthetic agent of high potency and low toxicity for use in many surgical procedures including

RUMENOTOMY
DISHORNING OF CATTLE

'Planocaine' is supplied as follows:
"V1" FORMULA (2 per cent solution without adrenaline) Rubber-capped bottles of 50 c.c.
"V2" FORMULA (2 per cent solution with adrenaline) Rubber-capped bottles of 50 c.c.
"T.10" TABLETS (containing adrenaline) Containers of 25 tablets

VETERINARY PRODUCTS M&B

TO THE PROFESSION ONLY

manufactured by

VA19 **MAY & BAKER LTD**

distributors

PHARMACEUTICAL SPECIALITIES (MAY & BAKER) LTD., DAGENHAM

GURR'S

STAINS and REAGENTS
for MICROSCOPY

FILTERED HISTOLOGICAL
PARAFFIN WAX
in 8 ounce tablets

For PERFECT SECTIONS
ALL MELTING POINTS

Packed in **7**, **14** and **28**lb. cartons

KNOWN and USED throughout THE WORLD

Please apply for Catalogue Y 15 and Literature

GEORGE T. GURR
136 NEW KINGS ROAD, LONDON, S.W.6

No connexion with any other business of similar name

H. K. LEWIS & Co. Ltd.

MEDICAL BOOKSELLERS and PUBLISHERS

BOOKSELLING DEPARTMENT

Large stock of BOOKS ON MEDICINE, SURGERY AND ALL BRANCHES OF SCIENCE. FOREIGN DEPARTMENT. Select stock available. Books not in stock obtained under licence. Catalogues on request.

OSTEOLOGY, ANATOMICAL DIAGRAMS, WALL CHARTS, etc.

SECOND-HAND DEPARTMENT 140 GOWER STREET

Large stock of second-hand recent editions.

STATIONERY DEPARTMENT Medical and Scientific Stationery.

LENDING LIBRARY. Medical and Scientific

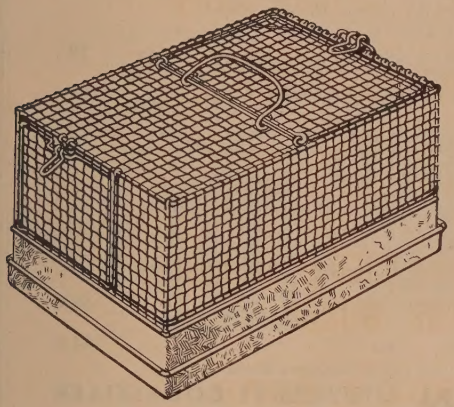
ANNUAL SUBSCRIPTION FROM ONE GUINEA.
Detailed Prospectus Post Free on Application.
Bi-monthly List of New Books and New Editions sent post free on request.

The Library Catalogue, revised to December 1049, is in preparation. pp. xii + 1152. To subscribers 17s. 6d. net.; To non-subscribers 35s. net., postage 1s. 4d.

PLEASE STATE INTERESTS WHEN WRITING

136 GOWER ST., LONDON, W.C.1
Telephone : EUSton 4282
Telegrams : Publicavit, Westcent, London

Complete Equipment for ANIMAL HOUSES



Specialists in
CAGES and RACKS for Rats, Rabbits, Guinea Pigs, etc.

On the Lists of M.R.C., A.R.C. and the Leading Hospitals and Universities.

Send us particulars of your requirements.

Deal with the actual Manufacturer.

S. RAMSEY & COMPANY LIMITED
Sebastian House . Sebastian Street . London, E.C.1

THE BRITISH VETERINARY JOURNAL

A Monthly Review of Veterinary Science

Editors :
T. DALLING M.A., M.R.C.V.S. & J. McCUNN M.R.C.V.S., L.R.C.P.

The BRITISH VETERINARY JOURNAL is the oldest journal of its kind in the country. It records the progress of Veterinary Science thoughout the world, but particularly in the British Isles and Commonwealth. *An indispensable journal to all who would keep abreast of progress in veterinary matters.*

Annual Subscription, 40s Single copies, 3s. 8d. post free

BAILLIÈRE, TINDALL, & COX
7 and 8 Henrietta Street, London, W.C.2

BOOKS ON APPROVAL

Below is a short list of some important English and American books recently published.

Any or all may be had entirely without obligation *ON APPROVAL FOR 7 DAYS* (Great Britain only.)

1. BARGER CARD (L. E.)
   Diseases and Parasites of Poultry. 4th Edition. 1949. **28/-**
2. FARRIS (E. J.)
   Care and Breeding of Laboratory Animals. 1951. ... **64/-**
3. HENNING (M. W.)
   Animal Diseases in South Africa. 2nd Edition. 1949. **70/-**
4. LANDER'S. *Veterinary Toxicology.* Revised by J. A. Nicholson. New Edition in Preparation.
5. LINTON (R. G.)
   Animal Nutrition and Veterinary Dietetics. 3rd Edition. 1950 ... ... **42/-**
6. MERCHANT (I. A.)
   Veterinary Bacteriology and Virology. 4th Edition. 1950. **72/-**
7. MORGAN (B. B.) *and* HAWKINS (P. A.)
   Veterinary Helminthology. 1949. ... ... **58/6**
8. MULLIGAN (R. M.)
   Neoplasms of the Dog. 1949. ... ... **31/6**

Write for our List of Veterinary Books.

JAMES THIN UNIVERSITY BOOKSELLER
NEW AND SECONDHAND
SOUTH BRIDGE, EDINBURGH.